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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

02075089.9

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Le Président de l'Office européen des brevets
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Blatt 2 der Bescheinigung
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Diagnostics and vaccines for Mycobacterium paratuberculosis infections

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SEE FOR THE ORIGINAL TITLE OF THE APPLICATION ,PAGE 1 OF THE DESCRIPTION.

P54977EP00

Title: Paramycobacterial diagnostics and vaccines.

The invention relates to the field of diagnosis, treatment and prevention of Johne's disease.

M. avium subsp. paratuberculosis is the causative agent of paratuberculosis or Johne's disease, a chronic granulomatous infection leading to disease in ruminants that is currently responsible for substantial worldwide economic losses for farmers and the dairy industry. The presence of this bacterium in pasteurized milk in combination with its suspected role in the development of Crohn's disease has raised concern regarding its potential health effects on the human population as well. Increased awareness of the problem has resulted in renewed urgency for the development of effective diagnostics and vaccines for control and eradication of paratuberculosis.

Mycobacterium avium comprises a large group of mycobacteria that can be divided into three subspecies, *M. .avium* subspecies *avium*, *M. avium* subspecies *silvaticum* and *M. avium* subspecies *paratuberculosis*. *M. avium* subspecies *avium* is widely distributed in the natural environment including soil and apparently healthy animals, as well as in birds and humans. *M. avium* subspecies *avium* isolates are opportunistic pathogens and generally cause infection and disease in immunocompromised hosts. The complete genomic sequence of *M. avium* subspecies *avium strain* 104 is currently being determined. *M. avium* subspecies *silvaticum* can produce a disease that resembles paratuberculosis in deer. Although most ruminants are infected with *M. avium subspecies paratuberculosis* before six months of age, clinical disease generally occurs only at four or five years of age. During this period, bacteria are believed to survive inside host cells, but extracellular episodes of infection in the lumen of the gastrointestinal tract – during which the bacterium becomes detectable in faeces – do also occur (with increasing frequency at later stages of infection). Currently available (immuno-) diagnostics against *M. avium subspecies paratuberculosis* have a relatively poor sensitivity, especially with respect to the detection of early or latent infection, and therefore are not effective as a tool for disease control. Whole cell Mycobacterial vaccines that are to some measure thought to be effective in freeing herds from clinical disease are

available, but these vaccines essentially interfere with the immunodiagnosis of bovine tuberculosis and do not inhibit transmission of disease.

To date several antigenic components of *M. avium subsp. paratuberculosis* have been identified. The antigenic molecules of *M.avium subsp. paratuberculosis* described previously comprise glycolipids and protein antigens identified with essentially monospecific early sera raised in small experimental animals. The cell wall glycolipid molecule lipoarabinomannan (LAM) was identified by its recognition by monoclonal antibodies raised against cell filtrate released by the bacterium, and has subsequently been purified and used for the development of a serodiagnostic ELISA (Mutharia et al., Infect. Immun. 1997. 65:387-394; Jark et al., 1997. Vet. Microbiol. 57:189-198). In addition, protein antigens with molecular weight of 14 kD (Olsen et al. Clin. Diagn. Lab. Immunol. 2001.8:797-801), 18 kD (bacterioferritin; Elsaghier et al. Clin. Exp. Immunol. 1992 90:503-508), 19 kD (AhpD; Olsen et al., Infect. Immun. 2000. 68:801-808), 24 kD (p24BCD; Elsaghier et al. Clin. Exp. Immunol. 1992 90:503-508), 30 kD(P30; Burrels et al.; Vet. Immunol. Immunpathol. 1995. 45:311-320), 34 kD (Gilot et al. J. Bact. 1993. 175:4930-4935; De Kesel et al J. Clin. Microbiol. 1993. 31: 947-954; Coetsier et al., Clin. Diagn. Lab. Immunol. 1998. 5: 446-451), 34.5 kD(Mutharia et al., Infect. Immun. 1997. 65:387-394),), a 35 kD protein (Dheenadhayalan and Chang, unpublished data), 38 kD (Elsaghier et al. Clin. Exp. Immunol. 1992 90:503-508), 44.3 kD (Mutharia et al., Infect. Immun. 1997. 65:387-394), 45 kD (AhpC; Olsen et al., Infect. Immun. 2000. 68:801-808), 65 kD (hsp65; Koets et al., Vet. Immunol. Immunopathol. 1999. 70:105-115), 70 kD (hsp70; Stevenson et al., 1991. Nucleic Acids Res. 19:4552; Koets et al., Vet. Immunol. Immunopathol. 1999. 70:105-115), and a superoxide dismutase molecule (Dheenadhayalan and Chang, unpublished data) have been identified and (partly) characterized. Only few of these have been evaluated for the development of diagnostics or vaccines (34 kD; ; Coetsier et al., Clin. Diagn. Lab. Immunol. 1998. 5: 446-451), and current diagnostics and vaccines are therefore still based on rather crude antigenic materials, that cannot always be used due to interference with immune diagnosis of other ruminant mycobacterial infections such as caused by *M. bovis* or *M. tuberculosis*, and generally do not inhibit transmission of disease.

The lipoarabinomannan (Mutharia et al., Infect. Immun. 1997. 65:387-394; Jark et al., 1997. Vet. Microbiol. 57:189-198) and 34 kD antigen (Gilot et al. J. Bact. 1993. 175:4930-4935; De Kesel et al J. Clin. Microbiol. 1993. 31: 947-954; Coetsier et al., Clin. Diagn. Lab. Immunol. 1998. 5: 446-451) have been described in DE19621488 and WO9216628, for use in diagnosis and vaccines. Several other molecules have been submitted for use in diagnostics, vaccines and therapeutics. These are proteins encoded on insertion sequence ISM-1 (EP0288306 and US 5225324;), the mycobacterial DAP molecule (US9523226), a 36 kD antigen (US5776692), a soluble antigen preparation (RU2118538), an extra cellular protein with an iron-reducing capacity (DE19728834), and an acylase (WO9949054).

The invention provides a method for obtaining a recombinant host cell comprising an antigenic polypeptide fragment of *M. avium subsp. paratuberculosis* and its encoding nucleic acid comprising providing a recombinant expression library of host-cells expressing *M. avium subsp. paratuberculosis* nucleic acid, followed by e.g. plating the library for plaques and immunoscreening said library and identifying said plaques with a serum obtained from a ruminant infected with *M. avium subsp. paratuberculosis* but not with *M. bovis*, said serum essentially obtained from a late stage of infection of said ruminant with said bacterium, the method further comprising selecting a host cell that expresses a fragment that is immunoreactive with said serum.

It is preferred, from both the diagnostic as well as the vaccine viewpoint, that said recombinant host cell expresses little or no other specific Mycobacterial antigens. A useful host cell is a host cell based on E. coli, as further explained in the detailed description herein, but other suitable hosts cells can be derived from the art as well. Especially when such a host cell is expressing little or no other Mycobacterial antigens, it can be used directly as a whole cell preparation for a vaccine or for diagnostic purposes, however, the relevant antigenic (poly)peptide or fragments can be at least partly purified from said host cell. Ease of purification is for example obtained when the relevant antigenic peptide is tagged, for example with a his-tag as further explained in the detailed description.

It is furthermore preferred that said ruminant was found to be naturally infected with *M. avium subsp. paratuberculosis*, but has no history of infection

with tuberculosis, brucellosis or leucosis, as evidenced by finding at least two *M. avium subsp. paratuberculosis* positive feces samples within an approximately two year long period before obtaining the test-serum for use in screening, and finding essentially no antibodies or other immune responses directed against agents causing tuberculosis, brucellosis or leucosis that are nor cross-reactive with paratuberculosis antigens. When such care is taken, a serum can be obtained that is very useful in immunoscreening for M. avium subsp. paratuberculosis antigens, being broadly reactive against relevant M. avium subsp. paratuberculosis peptide fragments but bearing essentially no or only little specific reactivity with tuberculosis, brucellosis or leucosis.

As said, such a serum need be essentially obtained from a late stage of infection of said ruminant with said bacterium in order to provide a serum directed to a broad repertoire of antigens of said bacterium, however, while sufficiently maintaining its specificity for the target. It is preferred that said ruminant is a cow, leading in a most preferred embodiment to a serum such as serum 3869, which was used to identify most of the sequences described in the detailed description. Said particular serum was derived at 18-12-1996 from a naturally infected cow which tested positive for the presence of *M. avium subsp. paratuberculosis* in feces samples obtained at 10.01.1995 and 29.09.1996.

In order to identify and characterize antigens in *M. avium subsp. paratuberculosis* for use in diagnostics, therapeutics and vaccines, we have constructed a genomic expression library using the lambda TripleEx expression vector according to the Clontech manual (PT3003-1) and Stratagene GigapackÆ III Gold Packaging manual. Briefly, bacterial genomic DNA isolated from *M. avium subsp. paratuberculosis* strain 316F was partially digested with *Tsp*509I and fragments of average size of 2.5 kilobasepairs, obtained by sucrose gradient centrifugation, were ligated to *Eco*RI-digested, dephosphorylated lambda TriplEx arms. The packaging reaction was carried out using Gigapack III Gold Packaging Extract and host strain E. coli XL1Blue (Clontech (S0924)). After plating of the library, immunoscreening of approximately 10^6 phage plaque's was carried out with 1) a positive bovine serum (designated as 3869) and 2) control monoclonal antibodies. This resulted in our hands in the selection of 125 positive lambda TriplEx recombinants. Hundred and seventeen of these 125 positive phage recombinants were successfully converted to plasmid (pTriplEx) recombinants using the protocol described in the Clontech manual (PT3003-1).

DNA sequencing of these 117 pTriplEx allowed these to be categorized into different antigen groups (designated SEQ 1-39) with each group expressing a different antigenic protein or fragment thereof. SEQ 1-16, 21-34 were based on recombinants isolated with serum 3869, SEQ 18 on recombinants isolated with monoclonal antibodies to FabG4, SEQ 19 on recombinants isolated with monoclonal antibodies to Hsp70, and SEQ 20 on recombinants isolated with monoclonal antibodies to Hsp65 and SEQ 35-39 on recombinants isolated with 5 respective monoclonal antibodies (13.67.1A; 10.65.3B; 13.67.2A; 10.32.3B; and 10.66.4B) directed to 5 antigenic molecules of M.avium subsp paratuberculosis. Blast searches against various data bases containing mycobacterial genomic information allowed further characterization of a number of the antigenic polypeptides and their encoding genes. Except for hsp65 and hsp70 heat shock protein antigens, none of the here provided antigenic fragments have so far been identified as a for *M. avium subsp. paratuberculosis* relevant antigen or figure among the already known antigens discussed above for *M. avium subsp. paratuberculosis*.

Using a method to obtain the desired host cells as provided herein, where special attention has been given to the selection of the serum used in immunoscreening thus results in an antigenic polypeptide fragment of *M. avium subsp. paratuberculosis,* obtainable from a host cell according to the invention, with hitherto unknown characteristics. In particular, the invention provides an antigenic polypeptide comprising a peptide fragment essentially derived from *M. avium subsp. paratuberculosis* bearing essentially no functional homology to *M. bovis and/or M. tuberculosis*. Here provided antigenic fragments have now been identified as an antigen relevant for *M.avium subsp. paratuberculosis* but do not figure among already known antigens discussed above for *M.avium subsp. paratuberculosis*, except for the hsp70 and hsp65 heat shock protein antigens. Using a method to obtain the desired host cells as provided herein, where special attention has been given to the serum used immunoscreening thus resulting in an antigenic polypeptide fragment of *M.avium subsp. paratuberculosis*, obtainable from a host cell according to the invention, with hitherto unknown characteristics, no other overlap with known antigenic fragments was found.

Having identified these antigenic polypeptide fragments, the invention provides novel antigens for diagnostic and vaccinial use in the field of *M. avium subsp. paratuberculosis* infections. In one embodiment, the invention provides an

antibody directed against a fragment provided herein. Such antibodies can specifically be used in detecting *M. avium subsp. paratuberculosis* antigens, but are also useful as competing antibody in a serologic assay such as an ELISA, or in other methods for testing samples for detecting *M. avium subsp. paratuberculosis* infections. In particular, the invention relates to antigenic peptides and uses thereof (SEQ 1-16, 18, 21-39; see enclosed listing below) of *M.avium subspecies paratuberculosis* that provide novel antigens for diagnostic, therapeutic and vaccinial use in the field of *M.avium subsp. paratuberculosis* infections. The antigenic peptides SEQ 9, 10, 12-14, 22-26, 29, 30, 34 and 38 have no known homologues in *M.tuberculosis/M.bovis*. The antigenic peptides SEQ 3-8, 11, 15, 16, 18, 21, 27, 28, 31-37, 39 display homologies with various ORFs predicted on the *M. bovis* and/or *M. tuberculosis* genome sequence, but none of these ORFs has thus far been identified as relevant antigenic molecules in these mycobacterial species. Both groups of antigenic peptides or fragments thereof therefore provide functionally specific *M.avium subsp. paratuberculosis* antigens for development of specific diagnostics, therapeutics and vaccines, that essentially do not crossreact or interfere with the immunodiagnosis of bovine tuberculosis. The antigenic peptides SEQ 1, 2, 19 and 20 are related to previously identified antigenic molecules of other mycobacteria including *M.bovis/M.tuberculosis* (resp. Erp or P34/P36 antigen; MPT53; hsp70; and hsp65). However, novel specific fragments within SEQ 1, 2 have been identified that are not present in *M.bovis/M.tuberculosis* and these are also useful for said purposes.

Thirty of the antigens (SEQ 1-16, 21-34) described herein have been identified using a serum sample from a cow with a late stage infection with *M. avium subsp. paratuberculosis* but with no evidence of being infected with *M. bovis*. Such late stage sera have the advantage of being directed to a broad range of *M.avium subsp. paratuberculosis* antigens that are generated during a live infection of a cow with said bacterium. Earlier identified antigens have been mainly identified by using monoclonal and polyclonal antibodies generated in mice and rabbits that have been immunised with killed (fractions) of *M.avium paratuberculosis*, and these antibodies therefore identify a different repertoire of antigens. Indeed, all 30 antigens (SEQ 1-16, 21-34) identified and characterised with the serum sample of a cow as described herein represent antigens that have not been described in these earlier studies. In addition, by using a monoclonal

antibody to fabG4 and 5 other antigenic molecules of M. avium subsp paratuberculosis, 6 novel antigens (SEQ18, 35-39) were identified and characterized as described herein, and by using monoclonal antibodies to the previously described hsp65 and hsp70 antigen homologues of these antigens (SEQ20 and SEQ19) were identified and characterized as described herein. Having identified the antigenic polypeptide fragments (SEQ 1-16, 18, 21-39; see enclosed listing below), the invention in particular provides an isolated nucleic acid selected from the group of SEQ 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38 or 39 and a polypeptide derived thereof as novel antigens for diagnostic, therapeutic and vaccinial use in the field of *M.avium subsp. paratuberculosis* infections. In a preferred embodiment, an isolated nucleic acid selected from the group of SEQ 9, 10, 13, 14, 22, 23, 24, 26, 29, 30, 34 or 38 is provided, bearing less than 65% homology with *M. tuberculosis* and/or *M. bovis*. The invention provides specific antigenic peptides or fragments thereof derived from *M.avium subsp. paratuberculosis* as provided herein for the development of specific and sensitive diagnostics that improve specific and early diagnosis of *M.avium subsp. paratuberculosis*. In one embodiment, the invention provides an antigenic peptide or a fragment derived thereof, or the use of a combination of such antigenic peptides or derived fragments, to specifically and sensitively detect antibodies in whole blood, serum, milk and other tissue and body fluid samples from animals or humans infected with *M.avium subsp. paratuberculosis*. Such peptides or derived fragments provide use for specific detection of antibodies in multistep serological assay formats such as an ELISA, but also in single step lateral flow "dipstick" formats. In combination with specific (monoclonal) antibodies directed against *M.avium subsp. paratuberculosis* such antigenic peptides or derived fragments also provide use in an (ELISA)-inhibition assay format where specific competing antibodies in above samples from humans and animals are detected. In another embodiment, the invention provides an antigenic peptide or derived fragments thereof, or a combination of such peptides and fragments, for use in cell-mediated assay formats where specific stimulation of a cell-mediated response - such as cell proliferation of secretion of specific cytokines such as interferon-gamma - in immune cells in or derived from whole blood or other body and fluid samples from animals or humans infected with *M.avium paratuberculosis* is detected after stimulation with said (combination

of) antigenic peptides or derived fragments in an *in vitro* cell culture system. The invention also provides use of the specific DNA sequences or fragments thereof derived from *M.avium subsp. paratuberculosis* as provided herein for the specific detection of *M.avium subsp. paratuberculosis* DNA in tissue samples infected with *M.avium subsp. paratuberculosis*. In one embodiment, oligonucleotide primers based on the specific DNA sequences or fragments herein provide design of a DNA-based amplification test such as a PCR and/or NASBA to specifically detect *M.avium susp. paratuberculosis* DNA in tissue samples from animals and humans infected with *M.avium susp. paratuberculosis*. In addition, specific DNA sequences or fragments thereof provide probes for the detection of *M.avium susp. paratuberculosis* DNA in tissue samples from animals and humans infected with *M.avium susp. paratuberculosis* by *in situ* hybridisation tests such as tests based on PNA and bDNA.

Furthermore, the invention provides use of an antigenic polypeptide or fragment thereof derived from *M. avium subsp. paratuberculosis* as provided herein for the production of a vaccine for the treatment or prevention of *M. avium subsp. paratuberculosis* infections, in particular for the development of a vaccine that does not interfere with the immunodiagnosis of bovine tuberculosis. In one embodiment, specific antigenic peptides or fragments herein, or a combination of such peptides and fragments, or hybrid molecules composed of specific peptides and fragments herein, provide use as subunit vaccines, in particular mixed with adjuvants such as oil-based emulsions, aluminium-based formulations, saponin-based formulations, or particle–based formulations are provided. Such vaccines are typically applied by the subcutaneous, intradermal, or intramuscular route and provide protective or therapeutic immunity to *M.avium subsp. paratuberculosis* in animals and humans, as said essentially without interfering with immunodiagnosis to bovine tuberculosis. In another embodiment, purified DNA molecules carrying DNA fragments encoding the antigenic peptides or fragments herein, or such vaccines carrying DNA fragments encoding hybrid molecules composed of (a combination of) antigenic peptides or fragments herein, provide use as DNA vaccines. DNA vaccines are typically applied by the intramuscular, intradermal or intranasal route and provide protective and therapeutic immunity to *M.avium subps. paratuberculosis* in animals and humans, essentially without interfering with immunodiagnosis to

bovine tuberculosis. In a third embodiment, live vaccine carriers such as *Salmonella* species expressing specific antigenic peptides or fragments herein, or expressing hybrid molecules composed of (a combination of) antigenic peptides or fragments provide live vaccines. Such vaccines are typically applied by oral route and provide protective and therapeutic immunity to *M.avium subps. paratuberculosis* in animals and humans, essentially without interfering with immunodiagnosis to bovine tuberculosis.

Figure 1. Immunblot displaying reactivity of serum samples from cows with a late stage infection with *M. avium subsp. paratuberculosis* B854 sonicate.

Samples

Lane M. Molecular weight markers

Lane 1-9. *M.avium subsp. paratuberculosis* B854 sonicate

Serum samples :

Lane 1.	Cow 't Gen	undiluted	27-01-99
2.	Cow' t Gen	1: 10	27-01-99
3.	Cow 284	undiluted	12-01-99
4.	Cow284	1: 10	12-01-99
5.	Cow744	undiluted	27-01-99
6.	Cow744	1:10	27-01-99
7.	Cow744	1:50/abs *E.coli*	27-01-99
8.	Cow3869	undiluted	18-12-96
9.	Cow3869	1:10	18-12-96

Table 1 *M. avium subsp. paratuberculosis* antigens identified from an λTRiplEX expression library of the genome of *M. avium subsp. paratuberculosis* 316F by serum antibodies from a healthy infected cow. Several antigens are represented by more than one independent clones selected from the library. Homology to known antigens from *M. avium paratuberculosis* (MAP) and to (predicted ORFs on) DNA contigs (ctg) of (partially completed) genome sequences of *M avium paratuberculosis*strain K10 (MAP), *M.avium avium* strain *104* (MAA), M.bovis strain AF 2122/97 (MBOV) and the *M.tuberculosis* strain H37RV (MTUB) genome sequence is indicated .

Ag	Number of clones	DNA Homo-logous to ctg in MAP Genome $	DNA Homo-logous to ctg in MAA Genome $	Antigenic Polypeptide homology to known AG or ORF on MAP genome $, *	Antigenic Poly peptide Homology to AG or ORF on ctg in MAA Genome$	Antigenic Polypeptide homology to MBOV Ag or ORF on ctg of MBOV genome$	Antigenic Polypeptide homology to MTUB Ag or ORFs predicted on MTUB genome$
1.	35	479 (100%)	22 (90%)	ORF on ctg 479 (100%)	ORF on ctg 22 (96%)	P36/P34 Ag (35 %) (on ctg272)	Rv3810/Erp/ PirG (35%)
2.	17	538 (100%)	65 (99%)	ORF on ctg 538 (100%)	ORF on ctg 65 (99%)	ORF on ctg 261 (82%)	Rv Mpt53/dsbE (82%), and DsbF (± 50 %)
3.	1	528 (100%)	93 (99%)	ORF on ctg 528 (100%)	ORF on ctg 93 (100%)	ORF on ctg 250 (71 %)	C-term of Rv1130 (77 %)
4.	1	498 (100%)	34 (100%)	ORF on ctg 498 (100%)	ORF on ctg 34 (100%)	ORF on ctg 252 (81%)	Rv2227 (84%)
5.	1	446 (97%)	13, (63%), 314 (63%),	ORF on ctg 446 (≥97%)	ORF on ctg 13, (46%), on ctg 314 (49%), and on ctg 156	ORF on ctg 276 (52 %), and on ctg272 (54%)	Rv3824c/ Rv3820c/Rv1182 (Pap A1, A2, A3 (52-54

			156 (60%)		(41%)		%)
6.	1	482 (99%)	98 (99%)	ORF on ctg 482 (≥99%)	ORF on ctg 98 (99%)	ORF on ctg 720 (73%)	Rv0999 (72%)
7.	3	436 (100%)	41 (100 %)	ORF on ctg 436 (100%)	ORF on ctg 41 (100 %)	ORF on ctg 232 (62%)	Rv1174c (73%)
8.	2	464 (99%)	27 (99%)	ORF on ctg 464 (≥99%)	ORF on ctg 27 (100%)	None	C-term Rv2255 (43%)
9.	1	None	None	None	None	None	None
10.	1	490 (100%)	None	ORF on ctg 490 (100%)	None	None	None
11.	3	517 (100%)	8 (99%)	ORF on ctg 517 (100%)	ORF on ctg 8 (100%)	> 40 ORFs (30-40%)	> 40 ORFS PE/PE-PGRS (30-40 %)
12.	1	536 (100%)	135 (98%)	ORF on ctg 536 (100%)	ORF on ctg 135 (100%)	ORF on ctg 280 (40%)	Rv 3800c (34 %)
13.	1	441 (100%)	110 (98%)	ORF on ctg 441 (100%)	ORF on ctg 110 (> 98%)	None	None
14.	1	469 (100%)	101 (100%)	ORF on ctg 469 (100%)	ORF on ctg 101 (100%)	None	None
15.	1	519 (99%)	218 (90%)	ORF on ctg 519 (≥ORF on ctg 99%)	ORF on ctg 218 (99%)	ORF on ctg 157 (92 %)	Rv1315/ MurA (92 %)
16.	1	467 (99%)	98 (98%)	467 (≥99%)	ORF on ctg 98 (99%)	ORF on ctg 194 (63%)	Rv1005c/ PabB (65%)
17.	1&	None	None	None	None	None	Parts of Rv0281

18.	7	529 (99%)	110 (99%)	ORF on ctg 529 (≥99%)	ORF on ctg 110 (99%)	ORF on ctg 262 (83%)	Rv0242c/FabG 4 (90%);
19.	1	469 (99%)	100 (99%)	Hsp70 (100%)	ORF on ctg 100 (99%)	ORF on ctg 260 (89%)	Rv0350/Hsp70 (89%)
20.	3	448 (100%)	116 (99%)	Hsp65 (100 %)	ORF on ctg 116 (99%)	ORF on ctg 283 (95 %)	Rv0440/Hsp65 (95%)
21.	1	471 (100%)	21 (99%)	ORF on ctg 471 (100%)	ORF on ctg 21 (99%)	ORF on ctg 244 (95%)	Rv0352/DNAJ (95%)
22.	1	538 (99%)	100 (95%)	ORF on ctg 538 (≥99%)	ORF on ctg 100 (≥95%)	None	None
23.	1	524 (100%)	364 (100%)	ORF on ctg 524 (100%)	ORF on ctg 364 (100%)	None	None
24.	1	526 (100%)	162 (98%)	ORF on ctg 526 (100%)	ORF on ctg 162 (≥98%)	None	None
25.	1	475 (100%)	174 (98%)	ORF on ctg 475 (100%)	ORF on ctg 174 (98%)	ORF on ctg284 (46%), and on ctg283 (45%)	Rv2263 (27%)
26.	1	None	174 (98%)	None	ORF on ctg 174 (≥98%)	None	None
27#.	1	404 (100%)	31 (99%)	InvA (100 %)	ORFs# on ctg 31 (100%)	None	#Rv2042c (69%)
28.	1	535 (100%)	95 (98%)	ORF on ctg 535 (100%)	ORF on ctg 95 (98%)	ORF on ctg 167 (66%)	Rv3463 (66%)
29.	1	527 (100%)	150 (70%)	ORF on ctg 527 (100%)	ORF on ctg 150 (≥70%)	None	None
30.	1	537 (100%)	38 (100%)	ORF on ctg 537 (100%)	ORF on ctg 38 (100%)	None	None

31.	1	539 (99%)	221 (98%)	ORF on ctg 539 (≥99%)	ORF on ctg 221 (98%)	ORF on ctg 249 (66%)	Rv1785c/cyt P450 (67%)
32.	1	478 (100%)	123 (98%)	ORF on ctg 478 (100%)	ORF on ctg 123 (100%)	ORF on ctg 247 (50%)	Rv1867 (40%)
33.	1	511 (99%)	12 (100%)	ORF on ctg 511 (≥99%)	ORF on ctg 12 (99%)	ORF on ctg 253 (74%), and ctg 282 (63%)	Rv0590 (74%)
34.	1	516 (98%)	301 (98%)	ORF on ctg 516 (≥98%)	ORF on ctg 301 (100%)	None	Rv3232 /PvdS (35 %)
35.	12	619 (99%)	137 (93%)	ORF on ctg 510 (>99%)	ORF on ctg 148 (98%)	ORF on ctg 213 (86%)	Rv0251c/fadE3 (86%)
36.	2	241 (100%)	223 (100%)	ORF on ctg 486 (100%)	ORF on ctg 189 (100%)	ORF on ctg 276 (61%)	Rv1326c/glgB (61%)
37.	2	196 (100%)	196 (99%)	ORF on ctg 389	ORF on ctg 917 (99%)	ORF on ctg 265 (84%)	Rv1928c (68%)
38.	2	414 (99%)	419 (99%)	ORF on ctg 531 (99%)	ORF on ctg 43 (99%)	None	None
39.	2	385 (98%)	386 (97%)	ORF on ctg 539 (98%)	ORF on ctg 18 (97%)	ORF on ctg 265 (70%)	LppE/Rv1881c (70%)

Two different ORFs - one homologous to InvA, and another homologous to Rv2042.

* derived from DNA homology

$ None means equal or lower than 40 % at protein level, or lower than 65 % at DNA level.

Detailed description.

Obtaining of sera.

The invention provides a serum obtained from a ruminant infected with *M. avium subsp. paratuberculosis* but not with *M. bovis,* said serum essentially obtained from a late stage of infection of said ruminant with said bacterium, the method further comprising selecting a host cell that expresses a fragment that is immunoreactive with said serum. It is preferred that said ruminant was found to be naturally infected with *M.avium subsp. paratuberculosis,* but has no history of infection with tuberculosis, brucellosis or leucosis, as evidenced by finding at least two *M. avium subsp. paratuberculosis* positive feces samples within an approximately two year long period before obtaining the test-serum for use in screening, and by obtaining the cows from herds that are granted an officially status declaring them free of tuberculosis, brucellosis or leucosis. When such care is taken, a serum can be obtained that is useful in immunoscreening for *M. avium subsp. paratuberculosis* antigens, being broadly reactive against relevant *M. avium subsp. paratuberculosis* peptide fragments but bearing no or at least no-detectable specific reactivity with tuberculosis, brucellosis or leucosis.

As said, such serum need be essentially obtained from a late stage of infection of said ruminant with said bacterium in order to provide a serum directed to a broad repertoire of antigens of said bacterium, however while sufficiently maintaining its specificity for the target. It is preferred that said ruminant is a cow, leading to a most preferred embodiment to a serum such as serum 3869, which was used to identify most of the sequences described in the detailed description. Said particular serum was derived at 19-12-1996 from a naturally infected cow which tested positive for the presence of *M. avium subsp. paratuberculosis* in feces samples obtained at 10.10.95 and 29.09.1996. The broad repertoire of antigens recognized by said serum 3869 is typically established by their reactivity to an whole cell preparation of *M. avium subsp. paratuberculosis* in an immunoblot (see Figure). Three other serum samples obtained at a late stage of infection from three other naturally infected cows displayed a similar broad repertoire in an immunoblot (see Figure). A similarly useful serum can experimentally be obtained from an specific-pathogen-free

(SPF) cow at a late stage of infection (typically at an age of four years or older) that has been experimentally infected within the first six months after birth. Experimental infection typically occurs orally - at three times a week for a period of four weeks - by using a tube to transport feces contaminated with *M. avium subsp. paratuberculosis* samples directly into the stomach.

With a serum as provided herein, the invention provides a tool for identifying or isolating hosts cells using a method for obtaining a recombinant host cell comprising an antigenic polypeptide fragment of *M.avium subsp. paratuberculosis* and its encoding nucleic acid comprising providing a recombinant expression library of host-cells expressing *M. avium subsp. paratuberculosis* nucleic acid, followed by e.g. plating the library for plaques and immunoscreening said library and identifying said plaques with said serum. Also the invention provides an isolated and/or recombinant nucleic acid comprising a sequence as identified hereunder or functional fragments thereof, derived from said host cell comprising said nucleic acid and an antigenic (poly)peptide. Also, the invention provides a nucleic acid that is essentially encoding an antigenic (poly)peptide as identified hereunder, or *M. avium subsp. paratuberculosis* specific variants thereof, and/or an *M. avium subsp. paratuberculosis* specific nucleic acid that is hybridizing under stringent conditions with a nucleic acid of which the sequence is herein provided. Also the invention provides a fragment encoded by said sequence that is hybridizing under stringent conditions with a nucleic acid of which the sequence is herein provided, thereby identifying a peptide fragment essentially derived from *M. avium subsp. paratuberculosis* bearing essentially a functional, or at least an antigenic, difference to a *M. bovis* and/or *M. tuberculosis* antigen.

Listing of sequences.

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 1 or functional fragments thereof, a host cell comprising said nucleic acid and an antigenic (poly)peptide and a fragment encoded by SEQ 1 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 1 comprises a DNA fragment of 1175 bp derived from recombinant phage 1821. Phage 1821 (and 34 other phages) encode *M. avium subsp. paratuberculosis* antigenic polypeptide 1.

DNA fragment of 1175 bp derived from recombinant phage 1821. Phage 1821 (and 34 other phages) encode *M. avium subsp. paratuberculosis* antigenic polypeptide 1.

AATTGCCTCACGATTCAATATCACCACTCTAGTAATAGGATTCCCACTCGT
ACCATCGACTGTGTGTGATTCCTGCCAGACAGCATCGGCGGGGCGCGCCG
ACACAACACATAGTCAGATAGAGGAGACTTCCGTGCCGAACCGACGCCGA
CGCAAGCTTTCGACAGCCATGAGCGCGGTCGCCGCCCTGGCAGTGGCGAG
TCCTTGCGCATACTTCCTTGTCTACGAATCGACGGCCGGCAACAAGGCGCC
CGAGCACCACGAGTTCAAGCAGGCCGCAGTGATGAGCGATCTGCCGGGCG
AGCTGATGGGTGCGCTGTCGCAGGGCCTGTCGCAGTTTGGGATCAACCTG
CCCCCGGTGCCCGCCCTGAGCGGCGGCGCCACCAGCACTCCCGGTCTGGC
CAGCCCCGGCCTGGGTAGCCCCGGCCTGGGCACGCCCGGCCTGGGAACGC
CGGGCCTGACCAATCCCGGTCTGACGAGCCCCGGTGCGACCAGTCCCGGC
CTGACCAGTCCCGGCCTGACCAGTCCTGGTTTGACCAGCCCCGGTCTGACC
AGCCCGGGTGCGGCGCCGACGACGCCCGGGCTCACCGCGCCCGGCGCGCT
GCCGACCACGCCGGGCGGCGGGGTCGCCACCCCCGGCGCCGGGCTCAACC
CCGCGCTGTCCAACCCCGGGCTGACCAGCCCGGCCGGGACGGCGCCGGGG
CTGGGCAGCCCGACCGTGGCGCCGAGTGAGGTGCCGATCGACTCCGGGGC
CGGCCTGGACCCGGGCGCCGGTGGCACGTACCCGATCCTGGGCGACCCGT
CGACCTTCGGTAACGCCTCGCCGATCGGCGGCGGTGGCACCGGTCTGGGC
GGCGGCTCGAGCTCGGGTGGCAGCGGCGGCCTGGTCAACGACGTGATGCA

AGCCGCCAACCAGCTCGGCGCGGGTCAGGCGATCGACCTGCTCAAGGGCC
TGGTGATGCCGGCGATCACGCAGGGCATGCACGGCGGCGCGGCCGCGGGT
GCTTTGCCCGGCGCGGCCGGTGCTCTGCCCGGCGCGGCCGGCGCCCTGCC
CGGTGCGGCCGGCGCCCTGCCGGGTGCGGCGGGCGCCGCGGGTGCGTTGC
CGGCGGCCGCCGGCGCCGCGCCGGCACTGCCCCCGGTCTAGACCTTTTCC
AAACCATCCACCAGACGGCACC

Antigenic polypeptide 1 encoded by SEQ 1 – length 336 amino acids

VPNRRRRKLSTAMSAVAALAVASPCAYFLVYESTAGNKAPEHHEFKQAAVM
SDLPGELMGALSQGLSQFGINLPPVPALSGGATSTPGLASPGLGSPGLGTPGLG
TPGLTNPGLTSPGATSPGLTSPGLTSPGLTSPGLTSPGAAPTTPGLTAPGALPTT
PGGGVATPGAGLNPALSNPGLTSPAGTAPGLGSPTVAPSEVPIDSGAGLDPGA
GGTYPILGDPSTFGNASPIGGGGTGLGGGSSSGGSGGLVNDVMQAANQLGAG
QAIDLLKGLVMPAITQGMHGGAAAGALPGAAGALPGAAGALPGAAGALPGA
AGAAGALPAAAGAAPALPPV

Comments.

1. The DNA sequence was obtained by sequence analysis of pTriplEx/1821. Sequencing of the 34 other pTriplEx recombinants showed a corresponding DNA sequence encoding antigenic peptide 1.

2. Antigenic polypeptide 1 is distantly (appr. 35 % amino acid identity) related to a smaller sized protein in *M.bovis* (secreted antigen P36/P34 precursor : Bigi et al., Infect. Immun. 1995. 63:2581-2586), and in *M.tuberculosis* (Erp protein : Lim et al., 1995. J. Bact. 177: 59-65; Berthet et. al. 1995. Microbiology 141:2123-2129; Berthet et. al. 1998. Science 282:759-762; Patent 6,010,855; pirG protein /Rv3810; Cole et al. 1998. Nature 393:537-544; and ID-SEQ16 in patent WO 99/09186).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 2 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 2 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 2 comprises a DNA fragment of 408 bp derived from phage 2221. Phage 2221 (and 16 other phages) encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 2 .

ACCGACGACCGCCTGCAATTCACCGCGACCACGCTCAGCGGCGCGCCGTTC
AACGGCGCCAGTCTGCAGGGCAAGCCCGCCGTGCTGTGGTTCTGGACGCCG
TGGTGCCCGTACTGCAACGCCGAGGCCCCGGGCGTGAGCCGGGTGGCCGCC
GCCAACCCGGGCGTCACCTTCGTCGGCGTCGCCGCCCACTCCGAAGTCGGC
GCCATGGCCAACTTCGTCTCCAAGTACAACCTGAACTTCACCACGCTCAACG
ACGCCGACGGCGCGATCTGGGCCCGCTACGGCGTGCCCTGGCAGCCCGCGT
ACGTGTTCTACCGGGCGGACGGCAGCTCCACCTTCGTCAACAACCCCACCTC
GGCGATGCCCCAGGACGAACTGGCCGCCCGGGTGGCGGCGCTGCGCTGA

Antigenic polypeptide 2 encoded by SEQ 2 –length 135 amino acids

TDDRLQFTATTLSGAPFNGASLQGKPAVLWFWTPWCPYCNAEAPGVSRVAA
ANPGVTFVGVAAHSEVGAMANFVSKYNLNFTTLNDADGAIWARYGVPWQPA
YVFYRADGSSTFVNNPTSAMPQDELAARVAALRstop

Comments.

1. The DNA sequence was obtained by sequence analysis of pTriplEx/2221. Sequencing of the 16 other pTriplEx recombinants showed a corresponding DNA sequence encoding antigenic peptide 2.

2. One region of 4 different amino acids (residues 63-66) and 21 other amino acid differences were identified in antigenic polypeptide 2 as compared to an ORF encoded by bp 43934-44342 on ctg 261 of *M.bovis* AF 2122/97 genome sequence; www.sanger.ac.uk/Projects/M_bovis) and as compared to related proteins in *M.tuberculosis* .(soluble secreted MPT52 protein Wiker et al. 1991: J. Gen.

Microbiol. 137:875-884; Wiker et. al. 1999. Microbial Pathogenesis 26:207-219; and DsbE protein/Rv2878c: Cole et al. 1998. Nature 393:537-544) . The *M.bovis* protein was found to be immunogenic following natural and experimental infection with *M.bovis* in cattle (Wiker et. al. 1999. Microbial Pathogenesis 26:207-219).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 3 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 3 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 3 comprises a DNA fragment of 171 bp derived from recombinant phage 2821. Phage 2128 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 3

AATTCCACGAACTGGCCGAGGGTGTCGTCACCGACGCCGAGCAGCAGCGGT
TCCTGTCGGTCGCCGAGGGCCTGCCCGCATTGCCGCCGGGCGCGGCGGGTG
AACTCAACATCGTGGTCGATCCGGCGGTGCTGGCCACCGCCCCGGCGATTC
CGGGCGGGATCTTCTGA

Antigenic open reading frame encoded by SEQ 3- length 55 amino acids

FHELAEGVVTDAEQQRFLSVAEGLPALPPGAAGELNIVVDPAVLATAPAIPGG
IFstop

Comments.

1.The 170 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/2128 derived from phage 2128.
2.Twenty three amino acid differences were identified in the polypeptide3 as compared to the C-terminal 56 amino acids of an ORF encoded by bp 7275-5707 on ctg 250 of the *M.bovis* AF 2122/97 genome sequence; www.sanger.ac.uk/Projects/M_bovis) and to the C-terminal end of an ORF in *M.tuberculosis* H37Rv (conserved hypothetical protein Rv1130: Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 4 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 4 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 4 comprises a DNA fragment of 153 bp derived from recombinant phage 4224. Phage 4224 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 4.

TTACCTCAGGGCCATGGCTATGTCTTCACCACCCGGGAACGTCCGGTGCGCT
CGGCCCGCGGCTGGTCCGCGGCGCCGGTACGCCACGGGGTCTCGGTGGTG
CGTTCCGGCCAGCGCTACGCGATGGGCTTGATCTTTCACGACGCCGCGTAG

Antigenic open reading frame encoded by SEQ 4 length 50 amino acids

LPQGHGYVFTTRERPVRSARGWSAAPVRHGVSVVRSGQRYAMGLIFHDAAsto
p

Comments.

1.The 153 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/4224 derived from phage 4224.

2. Nine amino acid differences were identified in the antigenic polypeptide 4 as compared to an ORF encoded by bp 34558–34726 on ctg 252 of *M.bovis* AF 2122/97 genome sequence;www.sanger.ac.uk/Projects/M_bovis), and 8 amino acid differences were identified as compared to the C-terminal 50 amino acids of the *M.tuberculosis* H37Rv hypothetical protein Rv2227 (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 5 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 5 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 5 comprises a DNA fragment of 551 bp derived from recombinant phage 3822. Phage 3822 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 5.

AATTGCTCGACGAGCGACAGACTGCCCGGTTCGAATCCGTTTGCCTGGCGG
CGGGCGCGCGATTCAGTGGCGGTGTGTTTGCCTGCGCCGCTCTCGTAGAAC
ACGAATTGACCGGCACCGAAACGTATTACGGAGTCATTCCCGTCGACATTCG
TCGCTCTCAGGACGAACTTGCGACAACGGGTTGGTTTGTCGGCTTTGTGCCG
CTCACCGTCTCTGTCGCGGTTCCTTCAGCAGACATCGTCCGCACCGCACAGG
CGTCCTTCGATTCGAACAAAGACCTTGCGAACGTTCCCGCCGAGCGCGTCG
NGGAGATGGCGCCGTGGCTGCGGATGCCTCAGCGGGGTGCTCCTTTGGTGT
TTTTCCTCGACGCCGGCGTGCCTCCCCTATCCGCTCTCGNTAATTCGCACTT
GGACGGTGCGAATGCCAGGCTCTACCACGACGGGAGGATTCCGTCTCAGGT
CGCCATCCGGGTTAATGGGCTTGAGAGCGAAACCCAAGTGATCGTGTTGCT
CCCGAACAATCCGATCGCCCGACAATTCGTGACCCAG

antigenic open reading frame encoded by SEQ 5–length 183 amino acids

LLDERQTARFESVCLAAGARFSGGVFACAALVEHELTGTETYYGVIPVDIRRS
QDELATTGWFVGFVPLTVSVAVPSADIVRTAQASFDSNKDLANVPAERVXEM
APWLRMPQRGAPLVFFLDAGVPPLSALXNSHLDGANARLYHDGRIPSQVAIR
VNGLESETQVIVLLPNNPIARQFVTQ

Comments.

1.The 551 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/3822 derived from phage 3822.

2.Four regions of 4 or more different amino acids (53-58, 75-78, 105-108, 113-116, and 133-136) and 60 other amino acid differences were identified in the antigenic

polypeptide 5 as compared to the C-terminal parts of ORFs encoded by bp 61939-62490 on ctg 276, by bp 33910-34455 on ctg 272 and by bp 26576-27115 on ctg 272 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis); and to the C-terminal parts of the *M.tuberculosis* H37Rv polyketide synthase associated proteins (pap) A1, A2 and A3 (Rv3824c, Rv3820c, and Rv1182; Cole et al. 1998. Nature 393:537-544). The full sequence of antigenic polypeptide 5 is still being determined.

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 6 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 6 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 6 comprises a DNA fragment of 917 bp derived from recombinant phage 5124. Phage 5124 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 6.

AATTGCGCGGAGTATTCGTACAGTTCGCCGGTGCGCGGGCCGCCGGGTGTC
AGCGCCTGCAGCACCCGGTGCACGCCCAACGTGCGCGCGCTGTCGATCTGC
GGCGCGTCGACCACTTGCACACCGCCGCGCAGTTGCGCACCGGAGAAGTTG
ACTTTGGCGCAGTCCTTCCCGGGGTCGTTGAACGGCAAGGCTTTTGAGGTCT
CGAGCGCGATCACCACGAAGCGGTTGCCGCGGCCCTCGGCGGTCACCGCGG
CCATGTTGCCCTGCAGATCGGCCGGCAGATCCGGCCCGCTCGCCACTTTCG
CGCACTGGGCGGGGTCGAAGGTCAGGCCGTCGGGCAGCTTGCGGCCGGCC
AGCAGCTTGGGGTCGATGCCGCGTTCGCCGGTGTCGCTGACCTTGTAGTCG
GGTCCGAAGCTGGGTTTCACGTCGGCGACCTTGGCGATGTCCACCTTCGCC
GGGTGAGTGGCCGATGAGCAGCCGGCCAGGACGCAGACAGCGGCCACCGC
GCGCAACAGCTTGGACATCGTGGCCAATCTACCCAAGCGGGTGGCTCAACT
GCGCAACGTGGACACCGTTTTGGCGAGCAGATCCGCGGCGAACTGCGGTGG
CAGCACCGGAACCGCCGCGCCGGGATCGGTGGTCAGGGTGGTGAAGGCGT
AGTAGTCGCCCAGATAGGCGATGAATGTGTAGCTGCGCGAATCGATTTCGG
TGCCCGATTCGACCGATGAGGTGATGTCGGCCACCATGCCCAGGGTTTCGA
CGCCGTCGATGTGTGAGCCGTCGGTGAGGCGGACGCGGACGGTGGTGTGC
CCGGCGGTCTCGGACCACTGCTGGCACGCGCCGAGCAGATCCCGCGGAAAG
TCCACGGGCTCCGGCAAAGCCACCACCACCGCGTCTACGATCCCGCCGCTG
CC

Antigenic open reading frame encoded by SEQ 6– length 184 amino acids.

ATRLGRLATMSKLLRAVAAVCVLAGCSSATHPAKVDIAKVADVKPSFGPDYK
VSDTGERGIDPKLLAGRKLPDGLTFDPAQCAKVASGPDLPADLQGNMAAVTA
EGRGNRFVVIALETSKALPFNDPGKDCAKVTFSGAQLRGGVQVVDAPQIDSA

RTLGVHRVLQALTPGGPRTGELYDYSAQ

Comments.

1. The 917 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/5124 derived from phage 5124.
2. Two regions of 4 or more different amino acids (29-33, 64-68) and 50 other amino acid differences were identified in antigenic polypeptide 6 as compared to an ORF encoded by bp 5116–5656 on ctg 720 of *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and to the related *M.tuberculosis* H37Rv hypothetical protein Rv0999 (Cole et al. 1998. Nature 393:537-544)..

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 7 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 7 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

Three phages (6821, 6822, and 6824) were isolated that carry SEQ 7, a DNA fragment of 314 bp encoding *M. avium subsp. paratuberculosis* antigenic polypeptide 7

AATTGGGCGTTGCGGTGGGCAGCGCGGCAGTGGCATTGACCGCCGCGGCCG
GTGTCGCATCCGCCGACCCCATGGACGCGATCATCAACACCACCTGCAACTA
CGGGCAGGTGATCGCCGCGCTGAACGCGTCCGACCCGGCGGCTGCCCAGCA
GCTGAACTCGTCGCCGATGGCGCAGTCCTACATCCAGCGGTTCCTGGCCTCC
CCGCCGGCGAAGCGTCAGCAGATGGCCCAGCAGATCCAGGGCATGCCGGCC
GCGCAGCAGTACATCAACGACATCAACCAGGTCGCGGTCACCTGTAACAACT
TCTGA

Antigenic polypeptide 7 encoded by SEQ7 –length 103 amino acids

LGVAVGSAAVALTAAAGVASADPMDAIINTTCNYGQVIAALNASDPAAAQQLS
SPMAQSYIQRFLASPPAKRQQMAQQIQGMPAAQQYINDINQVAVTCNNFstop

Comments.

1. A part of the DNA sequence was obtained by sequence analysis of 3 pTriplEx recombinants (pTriplEx/6821, pTriplEx/6822, and pTriplEx/6824) derived from the 3 phages 6821, 6822 , and 6824, respectively (bp 1-180). The full 314 basepair sequence of SEQ 7 has been obtained from *M. avium subsp. paratuberculosis* B854 genomic DNA using PCR with degenerate primers (based on the available *M.avium avium* 104 genome sequence; available through www.tigr.org) and subsequent DNA sequencing of the amplified product.

2. One region of 5 different amino acids (residues 91-95) and 37 other amino acid differences were identified in antigenic polypeptide 7 as compared to an ORF encoded by bp 21269–20955 on ctg 232 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and to the related *M.tuberculosis* H37Rv hypothetical protein Rv1174c (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 8 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 6 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 8 comprises a DNA fragment of 242 bp derived from recombinant phages 6823 and 7923. Phages 6823 and 7923 encode *M. avium subsp. paratuberculosis* antigenic polypeptide 8 .

AATTATGACCGTTAAAGTGTGTTCCGCCAAGGGGGTTGGGGGTGTGGCGCAG
TTATCAGCCGCCGGACCGTTCGCCACAATCAAGGCATGCGTGATCGCATGAC
GGCGACGCCGCGGGCCTGCAACCGGGACCGGGTCGCGCTGCAGGCCGTGC
ACTTTTTCATGGCCGACATGGAGGCCGGCATGGGCCCGTTCCTGGGCGTGC
TGCTGCAAAGCCGTGGCTGGACCACGGGCGCCATCGGC

Antigenic open reading frame encoded by SEQ8- length 30 amino acids

IMTVKVCSAKGVGVWRSYQPPDRSPQSRHAstop

Comments.

1. The 242 bp DNA sequence was obtained by sequence analysis of plasmids pTriplEx/6823 and pTriplEx/7923 derived from phage6823 and 7923, respectively.

2. Thusfar no significant homology was found in the antigenic polypeptide 8 as compared with ORFs encoded by the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis). One region of 5 different amino acids (1-5) and 10 other amino acid differences were identified in antigenic polypeptide 8 as compared the C-terminal 30 amino acids of the related *M.tuberculosis* H37Rv hypothetical protein Rv2255c (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 9 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 9 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 9 comprises a DNA fragment of 179 bp derived from recombinant phage 1221. Phage 1221 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 9:

AATTTTTCACTCAGTACAAATACCTATCAGCATGGAGAAACATGGAAGAGCA
ATTACAGCCAACACGTGTAGTCTTTTAAGAGTACACCAATAAATACCCATTT
GTGAAGGTTAATTTAATGCAACCCAGGCTGTTATCTGGAATAGTATATGTCG
CCAATTCAATCCATTAAGTAATT

Comments.

1. The 179 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/1221 derived from phage 1221.

2. The DNA sequence and the ORFS encoded by SEQ9 show no identity with the *M.bovis* AF 2122/97 genome and predicted ORF sequences (www.sanger.ac.uk/ Projects/M_bovis), nor with the genome and predicted ORF sequences of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 10 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 10 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 10 comprises a DNA fragment of 561 bp derived from recombinant phage 3226. Phage 3226 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 10.

AATTACCGCACGTCGTTCGAACGCTCAGGAGGCGACGCGGAGGGCCAGAGC
GTCGCTCTTGGTGATGAATATCCGAAGCGGTGTGGGGGCGGTCGAGATGAA
CGGTGACAGGAATCTTCCTGAAGGCTGGAAGGTCTATATCCGCGGTGGGTC
CGTGGAAGTGAAAGCGCCGGCGTCGGGCGGGACTTCTACCGGTTATCTACT
GACATCAGTTGAGGCCCGGCGTCTCGGTACAGAGATTCTTCGGGCGTTCGG
CCAGTGTGACGGTGACGGGTCGATTACCGCCCAGTACATGGACCCGATCCG
GTGGATCGTCTCGCTAACGGGCCGCAACGTGTGGTTGAGCGTCTCACCTGC
TGAACCAGATGGTCGATACGTGCTAAACGATGTCGAGTCAGGCGGGCTCGC
CGTGATGTTGTTGCAAGCGTCCGTGATGGTCGAGCAGCTTGACTCAGATGC
GGTGGATGGACCTGGAAGCGAACTGCTTGAGAAGGGCTTTCGGGAAATCCA
GGCCGGGACAGAGCGTCATTCAACTGAGATCCGTTGCTTGACAGACGAATT

Antigenic open reading frame encoded by SEQ 10- length 186 amino acids

ITARRSNAQEATRRARASLLVMNIRSGVGAVEMNGDRNLPEGWKVYIRGGSV
EVKAPASGGTSTGYLLTSVEARRLGTEILRAFGQCDGDGSITAQYMDPIRWIV
SLTGRNVWLSVSPAEPDGRYVLNDVESGGLAVMLLQASVMVEQLDSDAVDG
PGSELLEKGFREIQAGTERHSTEIRCLTDE

Comments.

1.The 561 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/3226 derived from phage 3226.

2. The DNA sequence and the antigenic polypeptide 10 shows no identity with the *M.bovis* AF 2122/97 genome and predicted ORF sequences (www.sanger.ac.uk/ Projects/M_bovis), nor with the genome and predicted ORF sequences of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 11 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 11 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 11 comprises a DNA fragment of 394 bp derived from recombinant phages 2922, 2925 and 7924. Phages 2922, 2925 and 7924 encode *M. avium subsp. paratuberculosis* antigenic polypeptide 11.

AATTGACGCACTGGACGGTGGTGCATCCCTCGCTGGTCGCCGCCAACCGCG
CCCGCCTGGCAATGCTGTTGGCCACCAACTTCTTCGGGATCAACTATCCGGC
CATCGCCGAAACCGAGGCCGAGTACCACGCCATGTGGGTGAACAACTCCGC
GGCGATGTACCGCTACGCGGCGACCTCGGCGACCGCGGTCAGGTTGCCCCG
GTTCACCGAGCCGCCTCAGGTGGCCAACCCGTCCGGGGTGAGCACCCAGGC
CGCGATGGTGCCCGCGACGAACGCCGCTGATTCCGGCACCCAGACCGGTGT
CGCCGGCACCCTGCAGGCCGCCTCCACCGCCTTCTTCGATCCCAACACTGGC
TGGTTCAAGTACTGGAGCACCTGGGGCAACCAATT

Antigenic open reading frame encoded by SEQ11– length 131 amino acids

LTHWTVVHPSLVAANRARLAMLLATNFFGINYPAIAETEAEYHAMWVNNSA
AMYRYAATSATAVRLPRFTEPPQVANPSGVSTQAAMVPATNAADSGTQTGVA
GTLQAASTAFFDPNTGWFKYWSTWGNQF

Comments.

1. The 394 bp DNA sequence was obtained by sequence analysis of plasmids pTriplEx/2922, pTriplEx/2925, and pTriplEx/7924 derived from phages 2922, 2925 and 7924 respectively.

2. Amino acid regions 1-9 and 92-131 of antigenic polypeptide 10 shows no significant identity with the genome and predicted ORF sequences of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), nor with the genome and predicted ORF sequences of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544). At least one region of 4 different amino acids (46-50) and 33 amino acid differences were identified in the region comprising the amino acids 10-91 of antigenic polypeptide 11 as compared to a variety of ORFs encoded by ctgs of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis); and to the *M.tuberculosis* H37Rv PPE proteins; i.p. Rv1789, Rv1808, Rv 1809, Rv2770, Rv 3136 : Cole et al. 1998. Nature 393:537-544). The full sequence of antigenic open reading frame encoded by SEQ 11 is still being determined.

3.The *M.tuberculosis* H37Rv PPE proteins consists of a large family of proteins of unknown function with subfamilies that carry specific proline-rich motifs (Cole et al. 1998. Nature 393:537-544). PPE proteins of *M.marinum* have been implicated as virulence factors affecting intracellular survival (Ramakrishnam et al., 2000. Science 288:1436-1437).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 12 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 12 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 12 comprises a DNA fragment of 188 bp derived from recombinant phage 3823. Phage 3823 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 12.

AATTCGATGGCGATCTCATGGGCGACGACGCCGCCGAAGGACCAGCCGAGG
AGGTTGTAGGGCCCGGTGGGATCAACGCCCTGGATCCGGTCGGCATAGTTT
TTCGCCATGTCGCGGATTGACGCCGGTTCGGCCTCGCCCCGCTGCAGGGAT
TGCTGAATCCCAATGATGGGGCAGCCCAGGTAATT

Antigenic open reading frame encoded by SEQ 12– length 62 amino acids

NYLGCPIIGIQQSLQRGEAEPASIRDMAKNYADRIQGVDPTGPYNLLGWSFGG
VVAHEIAIE

Comments.

1. The 188 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/3823 derived from phage 3823.
2. The amino acid region 1-40 of antigenic polypeptide12 shows no identity with the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/ Projects/M_bovis), nor with the predicted open reading frames of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544). The amino acid region 41-62 of antigenic polypeptide 12 shows 6 amino acid differences with an ORF encoded by bp 4798-4861 on ctg 280 of *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and with the related *M.tuberculosis* ORF pks13 (Rv3800c; Cole et al. 1998. Nature 393:537-544). The *M.tuberculosis* H37Rv pks13 is a probable polyketide synthase involved in cell wall biosynthesis.

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 13 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 13 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 13 comprises a DNA fragment of 57 bp derived from recombinant phage 91211. Page 91211 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 13.

AATTTCTTTGATTGCCCACTGATTTCGAGCTAGGGAGGACACTGATGACGGA
GAATT

Comments

1. The 57 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/91211 derived from phage 91211.
2. The DNA sequence and the ORFS encoded by SEQ13 show no identity with the *M.bovis* AF 2122/97 genome and predicted ORF sequences (www.sanger.ac.uk/ Projects/M_bovis), nor with the genome and predicted ORF sequences of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 14 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 14 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 14 comprises a DNA fragment of 84 bp derived from recombinant phage 2123. Phage 2123 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 14.

AATTTGCTGCCCCGCCCGCTCAGCGGTTCTCCGCTGGTCACCGGCCGACTAT
AACGCAGGCTCGGCAAATCGGCGGAGTGAATT

Comments.

1. The 84 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/2123 derived from phage 2123.
2. The DNA sequence and the ORFS encoded by SEQ14 show no identity with the *M.bovis* AF 2122/97 genome and predicted ORF sequences (www.sanger.ac.uk/ Projects/M_bovis), nor with the genome and predicted ORF sequences of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 15 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 15 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 15 comprises a DNA fragment of 692 bp derived from recombinant phage 2126. Phage 2126 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 15.

AATTGCGGCAGCCCGCGCACCACCGCGTGGTGCCCGTCGGTGCGGGCGTCG
GCGCCCAGCCGGATCATTTCCTCGACGAACCGGAAGCGCGCCTCGAACACG
TTCTCGGTGATCATCGAGGTGCCATCGGCGATCGACGCCAGGGCGATCGCC
ATCGGCTGCAGGTCGGTGGGAAATCCGGGAAACGGCAGGGTGGCCACGTTA
ACCGCCTTGGGCCGCTCGTACTGGGTCACCCGGAAGCTGTCGTCGGTCTGG
GTCACGGTGGCCCCGGCATCGTGCAACTTGTGCAGCACCACCTGCAGGTGG
GCGGGGTCGACGCCGGTCACCGAGATGTCCCCGCGGGTCATCGCCGCGGCG
ATGCCCCAGGTCGCGGCCACGATGCGGTCGCCGATCACCCGATGTTCGGTC
GGGTACAGCCGCGGCACGCCGGTGATGGTCATGGTCGGCGAACCCGCGCCC
TCGACCTGCGCGCCCATCTGGTTGAGCATCGTGCACAGATCCACCACGTCG
GGTTCGCGGGCCGCGTTGTGAATGGTGGTGACCCCCTCGGCCACCACCGCC
GGCATCAGGATGTTCTCGGTCGCCCCCACCGACGGGAACTCCAGCTGAATC
TCCGCGCCGCGCAACGTATCCGCTGCGCCACCACGCATCCGTGCTTGATGTT
TGCAGGTGGCGCCCAACTTGGCGCAAGC

Antigenic open reading frame encoded by SEQ15 – length 230 amino acids

ACAKLGATCKHQARMRGGAADTLRGAEIQLEFPSVGATENILMPAVVAEGVT
TIHNAAREPDVVDLCTMLNQMGAQVEGAGSPTMTITGVPRLYPTEHRVIGDR
IVAATWGIAAAMTRGDISVTGVDPAHLQVVLHKLHDAGATVTQTDDSFRVTQ
YERPKAVNVATLPFPGFPTDLQPMAIALASIADGTSMITENVFEARFRFVEEMI
RLGADARTDGHHAVVRGLPQ

Comments.

1. The 692 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/2126 derived from phage 2126.
2. The amino acid region 1-25 of antigenic polypeptide 15 shows no identity with ORFs of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), nor with the predicted open reading frames of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544)). The amino acid region 26-230 of antigenic polypeptide 15 displays 6 amino acid differences with an ORF encoded by bp 5397-6077 on ctg 157 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and with the *M.tuberculosis* H37Rv MurA protein (Rv1315; Cole et al. 1998. Nature 393:537-544). The *M.tuberculosis* H37Rv MurA protein is a UDP-N-Acetylglucosamine 1-carbovinyl transferase, an enzyme that functions in the cell wall peptidoglycan biosynthesis.

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 16 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 16 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 16 comprises a DNA fragment of 420 bp derived from recombinant phage 3827. Phage 3827 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 16.

AATTGCGTGACACGCACGCCTGGTACACCTCGCCGGCGCGGATGGCTTCCA
GGCAGGCCAGTACCCCGTCGCGGTGCGCGGCCCGGTCGGCGACGTCCCAGT
CGATCCGGCACGGGCGCGGCGGGCCGGCGGGCGCGGCCAGCGCGCCGGCG
AGCCACCGCGGCATCGGCGCGCCGGTGAGGCTCTCGTACCACCACTGCCCG
TCGCGGTCGCGGCGCAGGACGCAGTCCGTCCAGCCGCCGGCGGCCTCGGG
AATCCGGTTCGGCTTGGCGTCGGCGCCGCCGTCCGGATAGGACAGGTAACC
GATCCACCCGCCGCCCACATGCGGCGGCTGCGGTGTCCGCGCCGTGCCGAC
CGCGAACACGTCGCCGGGGGGGCACCGGACGCACCGGCAGGCTCGGCGCGA
TCACCGTCAGGGTGA

Antigenic open reading frame encoded by SEQ16 – length 139 amino acids

TLTVIAPSLPVRPVPPGDVFAVGTARTPQPPHVGGGWIGYLSYPDGGADAKPN
RIPEAAGGWTDCVLRRDRDGQWWYESLTGAPMPRWLAGALAAPAGPPRPCR
IDWDVADRAAHRDGVLACLEAIRAGEVYQACVSRN

Comments

1. The 420 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/3827 derived from phage 3827.
2. Two different regions of more than 4 amino acids (9-12, and 25-32) and 28 other amino acids differences were identified in antigenic polypeptide 16 as compared to an ORF encoded by bp 2428-2850 on ctg 194 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and to the related *M.tuberculosis* H37Rv pabB protein (Rv1005c; Cole et al. 1998. Nature 393:537-544). The *M.tuberculosis* H37Rv pabB protein is a probable p-aminobenzoate synthase. The full sequence of the antigenic open reading frame predicted by SEQ16 is still being determined.

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 18 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 18 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 18 comprises a DNA fragment of 1365 bp encoding antigenic peptide 18. Seven phages were isolated that carry 1323 bp of this DNA fragment encoding *M. avium subsp. paratuberculosis* antigenic polypeptide 18.

GTGGCTCCGAAGGTCTCGTCCGATCTGTTCTCGCAGATTGTCAATTCCGGTC
CTGGATCGTTTCTCGCCAAGCAGCTCGGCGTCCCGCAACCCGAGACGCTGC
GCCGCTACCGGCCCGGTGACCCGCCGCTGGCCGGGTCGCTGCTGATCGGCG
GCGAGGGCCGCGTGGTCGAGCCGCTGCGGGCGGCGCTGGCCAAGGACTAC
GACCTGGTCGGCAACAACCTGGGCGGGCGCTGGGCCGACCGGTTCGGCGG
GCTGGTCTTCGACGCCACCGGGATCACCACCCCGGAGGGCCTGAAGGGGCT
GTACGAGTTCTTCACCCCACTGCTGCGCAACCTGGGTCACTGCGCCCGCGTG
GTGGTGGTCGGCACCACGCCCGACGCCGCCGCCGGCCCGCACGAGCGGATC
GCCCAGCGCGCCCTGGAGGGCTTCACCCGGTCATTGGGCAAGGAGCTGCGC
AACGGCTCGACGGTGGCGCTGGTGTACCTGTCGCCGGCCGCCAAACCCGCC
GCGACGGGCCTGGAGTCGACCATGCGGTTCATCCTGTCGGCCAAGTCCGCC
TACGTCGACGGCCAGGTCTTCTACGTCGGCGAGGCCGACTCCACCCCCCCG
GCGGACTGGGAACGGCCGCTGGACGGCAAGGTCGCCATCGTGACCGGTGC
GGCCCGCGGAATCGGCGCCACGATCGCCGAGGTGTTCGCCCGCGACGGCGC
CCGCGTGGTCGCGATCGACGTGGAATCGGCCGCCGAGACGCTGGCCGAGAC
GGCCAGCCGGGTCGGCGGCACCGCGCTGTGGCTCGACGTCACCGCCCCCGA
CGCCGTCGACAAGATCACCGAGCACCTGCGCGAGCACCACGGCGGTCACGC
CGACATCCTGGTCAACAACGCCGGGATCACCCGCGACAAGCTGCTGGCCAA
CATGGACGACGCGCGCTGGGACGCCGTGTTGGCCGTGAATCTGCTTGCCCC
ACTTCGCCTTACCGAAGGGCTGGTGGGCAACGGCAGCATCGGCGAAGGCGG
CCGCATCGTCGGCCTTTCGTCGATGGCCGGCATCGCGGGCAACCGCGGCCA
GACCAACTACGCCACCACCAAGGCAGGCATGATCGGCCTCACCCAGGCGCT
GGCGCCGGAGCTCTACGACAAGGGCATCACCATCAACGCCGTCGCGCCGGG
ATTCATCGAGACCCAGATGACGGCCGCCATCCCGCTGGCCACCCGCGAGGT
GGGGCGCCGGATGAACTCGCTGCTGCAGGGCGGGCAGCCGGTGGACGTCG

CCGAAACCATCGCCTACTTCGCCAGCCCGGCGTCGAACGCGGTGACCGGCA
ACGTCATCCGGGTCTGCGGCCAGGCGATGCTGGGGGCATGA

Antigenic polypeptide 18 encoded by SEQ 18 – length 454 amino acids

VAPKVSSDLFSQIVNSGPGSFLAKQLGVPQPETLRRYRPGDPPLAGSLLIGGE
GRVVEPLRAALAKDYDLVGNNLGGRWADRFGGLVFDATGITTPEGLKGLYEF
FTPLLRNLGHCARVVVVGTTPDAAAGPHERIAQRALEGFTRSLGKELRNGST
VALVYLSPAAKPAATGLESTMRFILSAKSAYVDGQVFYVGEADSTPPADWERP
LDGKVAIVTGAARGIGATIAEVFARDGARVVAIDVESAAETLAETASRVGGTAL
WLDVTAPDAVDKITEHLREHHGGHADILVNNAGITRDKLLANMDDARWDAV
LAVNLLAPLRLTEGLVGNGSIGEGGRIVGLSSMAGIAGNRGQTNYATTKAGMI
GLTQALAPELYDKGITINAVAPGFIETQMTAAIPLATREVGRRMNSLLQGGQP
VDVAETIAYFASPASNAVTGNVIRVCGQAMLGAstop

Comments.

1. A part of the DNA sequence was obtained by sequence analysis of 7 pTriplEx recombinants derived from the 7 phages, respectively (bp 43-1365). The full basepair sequence of SEQ 18 has been obtained from *M. avium subsp. paratuberculosis* B854 genomic DNA using PCR with degenerate primers (based on the available *M.avium avium* 104 genome sequence; available through www.tigr.org) and subsequent DNA sequencing of the amplified product.
2. Fourty six different amino acids were identified in the antigenic polypeptide 18 as compared to an ORF encoded by bp 11096-12457 on ctg 262 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and as compared to the related *M.tuberculosis* H37Rv FabG4 protein (Rv0242c; Cole et al. 1998. Nature 393:537-544). The *M.tuberculosis* H37Rv FabG4 protein is a probable 3-OXOACYL-[ACYL-CARRIER PROTEIN] REDUCTASE.

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 19 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 19 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 19 comprises a DNA fragment of 1872 bp encoding antigenic polypeptide 19. One recombinant phage 10.12.1A was isolated that carries 1043 bp of this fragment encoding *M. avium subsp. paratuberculosis* antigenic polypeptide 19.

ATGGCTCGTGCGGTCGGTATCGACCTCGGGACCACCAACTCCGTCGTCGCA
GTCCTCGAGGGCGGTGACCCCGTCGTCGTCGCCAACTCCGAGGGCTCGCGG
ACCACCCCGTCCATCGTCGCGTTCGCCCGCAACGGCGAGGTGCTCGTCGGC
CAGCCCGCCAAGAACCAGGCGGTGACCAACGTCGACCGCACCATCCGTTCG
GTCAAGCGGCACATGGGCACCGACTGGTCCATCGAGATCGACGGCAAGAAA
TACACCGCTCAGGAGATCAGCGCCCGCGTGCTGATGAAGCTCAAGCGCGAC
GCCGAGGCCTATCTGGGTGAGGACATCACCGACGCGGTCATCACCGTACCG
GCGTACTTCAACGACGCCCAGCGTCAGGCGACCAAGGAAGCCGGCCAGATC
GCCGGCCTCAACGTGCTGCGCATCGTCAACGAGCCGACCGCGGCCGCGCTG
GCCTACGGCCTGGACAAGGGCGAGAAGGAGCAGACCATCCTGGTCTTCGAC
CTCGGCGGCGGCACGTTCGACGTTTCGCTGCTCGAGATCGGCGAGGGTGTG
GTCGAGGTCCGCGCCACCAGCGGTGACAACCAACTCGGTGGCGACGACTGG
GACGACCGGATCGTCAACTGGCTGGTCGACAAGTTCAAGGGCACCAGCGGC
ATCGACCTGACCAAGGACAAGATGGCCATGCAGCGGCTGCGTGAGGCCGCC
GAGAAGGCCAAGATCGAGTTGTCCAGCTCGCAGAGCACCTCGATCAACCTG
CCCTACATCACCGTCGACGCGGACAAGAACCCGCTGTTCCTCGACGAGCAG
CTGACCCGCGCCGAATTCCAGCGCATCACCCAGGATCTGCTGGACCGCACC
CGTCAGCCGTTCAAGTCGGTGATCGCCGACGCCGGCATCTCGGTGTCCGAC
ATCGACCACGTGGTGCTGGTGGGTGGTTCCACCCGGATGCCCGCGGTGACC
GACCTGGTCAAGGAACTCACCGGCGGCAAGGAGCCCAACAAGGGCGTCAAC
CCCGACGAGGTTGTCGCGGTGGGTGCCGCCCTGCAGGCCGGTGTGCTTAAG
GGCGAGGTGAAAGACGTTCTGCTGCTTGACGTTACGCCGCTGAGCCTGGGT
ATCGAGACCAAGGGTGGCGTGATGACCAAGCTGATCGAACGCAACACCACC
ATCCCGACCAAGCGGTCCGAGACGTTCACCACGGCCGACGACAACCAGCCG

TCGGTGCAGATCCAGGTGTATCAGGGTGAGCGCGAAATCGCCGCGCACAAC
AAGCTGCTCGGCTCCTTCGAGCTGACCGGAATTCCGCCGGCGCCCCGCGGC
GTGCCGCAGATCGAGGTCACCTTCGACATCGACGCCAACGGCATCGTGCAC
GTCACCGCCAAGGACAAGGGCACCGGTAAGGAGAACACGATCAAGATCCAG
GAGGGCTCCGGCCTGTCCAAGGAGGAGATCGACCGGATGATCAAGGACGCC
GAGGCGCACGCCGAGGAGGACCGCAAGAGGCGCGAGGAAGCCGACGTCCG
CAACCAAGCGGAATCGCTTGTCTACCAGACGGAGAAGTTCGTCAAGGACCA
GCGCGAGGCCGAGGGCGGCTCGAAGGTTCCCGAGGAGACGCTGTCCAAGG
TCGACGCCGCGATCGCCGACGCCAAGACGGCCCTGGGCGGCACCGACATCA
CCGCGATCAAGTCGGCGATGGAGAAGCTCGGCCAGGAGTCGCAAGCGCTGG
GACAGGCAATCTACGAGGCCACCCAGGCCGAGTCCGCCCAGGCTGGCGGGC
CGGACGGTGCCGCGGCCGGCGGCGGGTCCGGATCCGCCGACGATGTTGTG
GACGCGGAGGTGGTCGACGATGACCGGGAGTCCAAGTGA

Antigenic polypeptide 19 encoded by SEQ 19 – length 623 amino acids

MARAVGIDLGTTNSVVAVLEGGDPVVVANSEGSRTTPSIVAFARNGEVLVGQP
AKNQAVTNVDRTIRSVKRHMGTDWSIEIDGKKYTAQEISARVLMKLKRDAEA
YLGEDITDAVITVPAYFNDAQRQATKEAGQIAGLNVLRIVNEPTAAALAYGLD
KGEKEQTILVFDLGGGTFDVSLLEIGEGVVEVRATSGDNQLGGDDWDDRIVN
WLVDKFKGTSGIDLTKDKMAMQRLREAAEKAKIELSSSQSTSINLPYITVDAD
KNPLFLDEQLTRAEFQRITQDLLDRTRQPFKSVIADAGISVSDIDHVVLVGGST
RMPAVTDLVKELTGGKEPNKGVNPDEVVAVGAALQAGVLKGEVKDVLLLDV
TPLSLGIETKGGVMTKLIERNTTIPTKRSETFTTADDNQPSVQIQVYQGEREIA
AHNKLLGSFELTGIPPAPRGVPQIEVTFDIDANGIVHVTAKDKGTGKENTIKIQ
EGSGLSKEEIDRMIKDAEAHAEEDRKRREEADVRNQAESLVYQTEKFVKDQR
EAEGGSKVPEETLSKVDAAIADAKTALGGTDITAIKSAMEKLGQESQALGQAI
YEATQAESAQAGGPDGAAAGGGSGSADDVVDAEVVDDDRESKstop

Comments.

1. A part of the DNA sequence was obtained by sequence analysis of pTriplEx/10.12.1A recombinants derived from the phage 10.12.1A (bp 830-1872).

The full base pair sequence of SEQ 19 has been obtained from *M. avium subsp. paratuberculosis* B854 genomic DNA using PCR with degenerate primers (based on the available *M.avium paratuberculosis* hsp70 gene sequence; Stevenson et al., 1991. Nucleic Acids Res. 19:4552) and subsequent DNA sequencing of the amplified product.

2. Two amino acid differences were identified in antigenic polypeptide 19 as compared to the *M. avium paratuberculosis* HSP70 protein (Stevenson et al., 1991. Nucleic Acids Res. 19:4552). Fourty four amino acid differences were identified in antigenic polypeptide 19 as compared to an ORF encoded by bp 23807-25567 on ctg 260 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and to the related *M.tuberculosis* H37Rv hsp70 protein (Rv0350; Cole et al. 1998. Nature 393:537-544).

3. Patents exist describing hsp70 molecules from other (myco)bacterial species, eg.US 5.830,475 and 5,723,296.

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 20 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 20 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 20 comprises a DNA fragment of 1626 bp encoding antigenic polypeptide 20. Three phages (10.20.3B, 13.53.3A.2, and 10.69.4B) were isolated that carry 1615 bp of this fragment encoding *M. avium subsp. paratuberculosis* antigenic polypeptide 20.

ATGGCCAAGACAATTGCGTACGACGAAGAGGCCCGTCGCGGCCTCGAGCGG
GGGCTCAACGCCCTCGCCGACGCGGTAAAGGTCACGTTGGGCCCCAAGGGT
CGCAACGTCGTCCTGGAGAAGAAGTGGGGTGCCCCCACGATCACCAACGAT
GGTGTGTCCATCGCCAAGGAGATCGAGCTGGAGGACCCGTACGAGAAGATC
GGCGCCGAGCTGGTCAAGGAAGTCGCCAAGAAGACCGACGACGTCGCCGGT
GACGGCACGACGACGGCCACGGTGCTCGCCCAGGCGTTGGTCCGCGAGGG
CCTGCGCAACGTCGCGGCCGGCGCCAACCCGCTGGGTCTCAAGCGCGGCAT
CGAGAAGGCCGTCGAGAAGGTCACCGAGACCCTGCTCAAGTCGGCCAAGGA

GGTCGAGACCAAGGACCAGATCGCTGCCACCGCGGCCATCTCCGCGGGCGA
CCAGTCGATCGGCGACCTGATCGCCGAGGCGATGGACAAGGTCGGCAACGA
GGGCGTCATCACCGTCGAGGAGTCCAACACCTTCGGCCTGCAGCTCGAGCT
CACCGAGGGTATGCGGTTCGACAAGGGTTACATCTCGGGCTACTTCGTCAC
GGACGCCGAGCGTCAGGAAGCGGTCCTCGAGGACCCGTTCATCCTGCTGGT
CAGCTCCAAGGTCTCGACCGTCAAGGACCTGCTGCCGCTGCTGGAGAAGGT
CATCCAGGCCGGCAAGCCGCTGCTGATCATCGCCGAGGACGTCGAGGGCGA
GGCCCTGTCCACCCTGGTCGTCAACAAGATCCGCGGCACCTTCAAGTCGGT
GGCCGTCAAGGCGCCCGGCTTCGGCGACCGCCGCAAGGCGATGCTTCAGGA
CATGGCCATCCTCACCGGCGGCCAGGTCATCAGCGAAGAGGTCGGCCTGTC
GCTGGAGAGCGCCGACATCTCGCTGCTCGGTAAGGCCCGCAAGGTCGTCGT
CACCAAGGACGAGACCACCATCGTCGAGGGCGCCGGTGACTCCGACGCCAT
CGCCGGCCGGGTGGCCCAGATCCGCACCGAGATCGAGAACAGCGACTCCGA
CTACGACCGCGAGAAGCTGCAGGAGCGGCTGGCCAAGCTGGCCGGCGGCG
TGGCGGTGATCAAGGCCGGCGCCGCGACCGAGGTCGAGCTCAAGGAGCGC
AAGCACCGCATCGAGGACGCGGTCCGCAACGCCAAGGCGGCCGTGGAGGA
GGGCATCGTCGCCGGCGGTGGCGTGGCCCTGCTGCACGCGATCCCGGCTCT
GGACGAGCTGAAGCTCGAGGGCGAAGAGGCGACCGGCGCCAACATCGTCC
GGGTGGCCCTCGAGGCTCCGCTGAAGCAGATCGCCTTCAACGGTGGCCTGG
AGCCCGGCGTGGTGGCCGAGAAGGTCCGCAACTCGCCCGCCGGTACCGGCC
TCAACGCCGCCACCGGTGAGTACGAGGACCTGCTCAAGGCCGGCATTGCCG
ACCCGGTGAAGGTCACCCGCTCGGCGCTGCAGAACGCGGCGTCCATCGCGG
GGCTGTTCCTGACCACCGAGGCGGTCGTCGCCGACAAGCCGGAGAAGGCGG
CCGCTCCCGCGGGCGACCCGACCGGCGGCATGGGCGGCATGGACTTCTGA

Antigenic polypeptide 20 encoded by SEQ20– length 541 amino acids

MAKTIAYDEEARRGLERGLNALADAVKVTLGPKGRNVVLEKKWGAPTITND
GVSIAKEIELEDPYEKIGAELVKEVAKKTDDVAGDGTTTATVLAQALVREGLR
NVAAGANPLGLKRGIEKAVEKVTETLLKSAKEVETKDQIAATAAISAGDQSIG
DLIAEAMDKVGNEGVITVEESNTFGLQLELTEGMRFDKGYISGYFVTDAERQ
EAVLEDPFILLVSSKVSTVKDLLPLLEKVIQAGKPLLIIAEDVEGEALSTLVVN
KIRGTFKSVAVKAPGFGDRRKAMLQDMAILTGGQVISEEVGLSLESADISLLG
KARKVVVTKDETTIVEGAGDSDAIAGRVAQIRTEIENSDSDYDREKLQERLAK
LAGGVAVIKAGAATEVELKERKHRIEDAVRNAKAAVEEGIVAGGGVALLHAIP

ALDELKLEGEEATGANIVRVALEAPLKQIAFNGGLEPGVVAEKVRNSPAGTG
LNAATGEYEDLLKAGIADPVKVTRSALQNAASIAGLFLTTEAVVADKPEKAAA
PAGDPTGGMGGMDFstop

Comments.

1. A part of the DNA sequence was obtained by sequence analysis of 3 pTriplEx recombinants (pTriplEx/10.20.3B, pTriplEx/13.53.3A.2, and pTriplEx/10.69.4B) derived from the 3 phages 10.20.3B, 13.53.3A.2, and 10.69.4B). The full 1626 basepair sequence of SEQ 20 has been obtained from *M. avium subsp. paratuberculosis* B854 genomic DNA using PCR with degenerate primers (based on the available *M.avium avium* 104 genome sequence; available through www.tigr.org) and subsequent DNA sequencing of the amplified product.
2.Seven and two amino acids differences were identified in antigenic polypeptide 20 as compared hsp60 sequences of *M.avium paratuberculosis* strain Linda described by el-Zaatari et al. (Clin. Diagn. Lab. Immunol. 2: 657-664, 1995; Accession numbers AAA996679 and P42348) One region of 6 different amino acids (528-533) and 26 other amino acid differences were identified in antigenic polypeptide 20 as compared to an ORF encoded by bp 78660-77041 on ctg 283 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and to the related *M.tuberculosis* H37Rv hsp60 (GroEL2/Rv0440; Cole et al. 1998. Nature 393:537-544)

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 21 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 21 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 21 comprises a DNA fragment of 364 bp derived from recombinant phage 8921. Phage 8921 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 21.

AATTCAAGGAGATCAGCGTCGCCTACGAGGTGCTCAGCGACCCGGAGAAAC
GGCGGATCGTCAACCTGGGCGGTGATCCGCTGGAGGGGGCGGCCGCGGCG
GGCGGCGGCTTCGGAGGGTTCGGCGGCCTCGGCGATGTGTTCGAGGCCTTT
TTCGGGGGCGGCTTCGGCGGCGGCACCACCTCCCGCGGCCCGATCGGGCG
GGTCCGGCCGGGCTCGGATTCGCTGCTGCGGATGCGGCTCGACCTCGAGGA
GTGCGCCACCGGGGTGACCAAGCAGGTCACCGTCGACACCGCCGTGCTGTG
CGACCGCTGCCACGGCAAGGGCACCAACGGCGACTCCGCCCCGGTGCCATG
CGACACCTG

The antigenic open reading frame encoded by SEQ 21;length 120 amino acids

FKEISVAYEVLSDPEKRRIVNLGGDPLEGAAAAGGGFGGFGGLGDVFEAFFG
GGFGGGTTSRGPIGRVRPGSDSLLRMRLDLEECATGVTKQVTVDTAVLCDRC
HGKGTNGDSAPVPCDT

Comments.

1. The 364 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/8921 derived from phage 8921.
2. Ten different amino acids were identified in antigenic polypeptide 21 as compared to an ORF encoded by 19565-19924 on ctg 244 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and to the related *M.tuberculosis* H37Rv DNAJ protein (Rv0352; Cole et al. 1998. Nature 393:537-544). DNAJ acts with GrpE to stimulate DnaK ATPase (hsp70) activity.

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 22 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 22 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

DNA fragment of 240 bp derived from recombinant phage 5524. Phage 5524 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 22.

AATTCAACGAGATTCCTCGCGAGGGGATCCGCACCTTGGCGGTTCAAGTATC
AGCAATCGTCCGCAGCAGAGTTAGCGGCAATTCATGGCAACCATCAGAGCC
AACGTTCTCAACACACCTTGGTTCACCAAACGTCCAGATAGGTTCGTCAGCA
TCGAGAAGGTCAACACGTTCTGAGCCNCGGATCAGGTTGCCGCGCAACGGG
ATTCGTCAGCGGCCGAAGTCCGACGGCCGGAATT

Comments.

1. The 240 bp DNA sequence was obtained by sequence analysis of plasmids pTriplEx/5524.2 and pTriplex/5524.3 derived from phage 5524.
2. The DNA sequence and the ORFS encoded by SEQ22 show no identity with the *M.bovis* AF 2122/97 genome and predicted ORF sequences (www.sanger.ac.uk/ Projects/M_bovis), nor with the genome and predicted ORF sequences of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 23 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 23 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 23 comprises a DNA fragment of 61 bp derived from recombinant phage 4223. Phage 4223 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 23.

AATTCGGTGATGAAGGCGGCGCGCTGCGCCACCGGGATCTCCTGCAACTGC
TTGAGTAATT

Antigenic open reading frame encoded by IDSEQ 23 – length 19 amino acids

LLKQLQEIPVAQRAAFTTE

Comments.

1. The 61 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/4223 derived from phage 4223.
2. The DNA sequence and the antigenic polypeptide 23 show no identity with the *M.bovis* AF 2122/97 genome and predicted ORF sequences (www.sanger.ac.uk/ Projects/M_bovis), nor with the genome and predicted ORF sequences of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 24 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 24 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 24 comprises a DNA fragment of 94 bp from recombinant phage 7926. Phage 7926 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 24.

AATTACGTCACTGTGACGCCGCGATCGGTGCGGGCCCGAATCCGCCCGGCC
GGTGCCGGGTGGCTCGGCGAAATCGCATGTGCACCAACAAATT

Comments.

1. The 94 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/7926 derived from phage 7926.
2. The DNA sequence and the ORFS encoded by SEQ24 show no identity with the *M.bovis* AF 2122/97 genome and predicted ORF sequences (www.sanger.ac.uk/ Projects/M_bovis), nor with the genome and predicted ORF sequences of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 25 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 25 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 25 comprises a DNA fragment of 83 bp derived from recombinant phage101022. Phage 101022 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 25 .

AATTGGCCGGCCAAGCGGGCCGGGACCGCCGCCGCGCTGTGGGAGCTGTCC
GAGGAACTGACCGGGACCAAGTTTCCGCTCTGA

Antigenic open reading frame encoded by SEQ 25 – length 27 amino acids

NWPAKRAGTAAALWELSEELTGTKFPLstop

Comments.

1. The 83 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/101022 derived from phage 101022.
2. The antigenic polypeptide 25 shows no identity with ORFS predicted on the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis). The amino acid region 1-12 of antigenic polypeptide25 shows no identity with the genome and predicted open reading frames of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544). Eight amino acid differences were identified in the amino acid region 13-27 of antigenic polypeptide 25 as compared to the C-terminal 17 amino acids of a related *M.tuberculosis* possible oxidoreductase protein (Rv2263; Cole et al. 1998. Nature 393:537-544), and to the C-terminal 16 amino acids of *M.tuberculosis* proteins Rv0068 and Rv0439c (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 26 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 26 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 26 comprises a DNA fragment of 59 bp derived from recombinant phage 5921. Phage 5921 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 26.

AATTCCAACGGCGCGTGTGCCNCGGCGCCCGCNCNGACTNCCTATCGGNGA
ACGCAATT

Comments.

1. The 59 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/5921 derived from phage 5921.
2. The DNA sequence and the ORFS encoded by SEQ26 show no identity with the *M.bovis* AF 2122/97 genome and predicted ORF sequences (www.sanger.ac.uk/ Projects/M_bovis), nor with the genome and predicted ORF sequences of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 27 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 27 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 27 comprises a DNA fragment of 374 bp derived from recombinant phage 6923. Phage 6923 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 27.

AATTGGCCGGGGCCGCCGGCCTCGTCGTCGGGCTGCTGGGGTTGCCGGCGC
TGGGAATCGCCGCGGCCGCCGGGCTGGTGGTGTTCTTCGTCGGTGCGGTGC

TGACGCACCTGCGGGCCGGCGTGCTGTACAACATCGCGTTTCCCGGGGCCT
ACCTGTGTCTGTCCGCGGCATCGCTGGCCTGGATGGCTCTGCGCTGAATCG
GGCGCGAGGTCAGGCCGGGAAGTAGCGGATCCGGTTGATCGCGTTGGCCCG
CCAGGCCACATCGGCGAACATGATGGCGCGGCCGTGACCCGAATGCAGCGA
GACCGCCACGGCGGGGCCGGCGCTGTTCATCTTGGTGAGGCCCGCCCCCGC
CAACTGTTCGGCCAATT

antigenic open reading frame 1 encoded by SEQ 27– length 65 amino acids

LAGAAGLVVGLLGLPALGIAAAAGLVVFFVGAVLTHLRAGVLYNIAFPGAYLC
LSAASLAWMALRstop

Antigenic open reading frame 2 encoded by SEQ 27– length 52 amino acids

LAEQLAGAGLTKMNSAGPAVAVSLHSGHGRAIMFADVAWRANAINRIRYFPA
stop

Comments.

1. The 374 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/6923 derived from phage 6923.
2. The antigenic open reading frame 1 of SEQ27 is identical to the *M.paratuberculosis* InvA protein (Bull et al., Microbiology 2000. 146:2185-2197). No significant homology was identified between the antigenic open reading frame 1 encoded by SEQ 27 as compared to ORFs encoded by the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), nor with the predicted open reading frames of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544). Sixteen different amino acids were identified in antigenic open reading frame 2 of SEQ27 as compared to an ORF encoded by bp 16729-16884 on ctg 246 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and to the related open reading frame Rv2042c of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 28 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 28 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 28 comprises a DNA fragment of 215 bp derived from recombinant phage 7922. Phage 7922 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 26.

AATTACGTGCACAACTGGCTGCGGCTGGGGTTCACCGAGGCCGACGTGCGC
CCACCGGGCAGCGACCGGCTGATCGACGCGGTGATCAGCTACGGCACCCCG
CAGGCCGTCGCGGCGCGACTGCGCGAGCATCTGGACGCCGGGGCCGACCA
CGTGGCGATCCAGGTGCTGGGCGGGGATTCCGAGGAGACGCTGCTGCCCGC
GCTGACCGAATT

Antigenic open reading frame encoded by SEQ 28 – length 71 amino acids

NYVHNWLRLGFTEADVRPPGSDRLIDAVISYGTPQAVAARLREHLDAGADHV
AIQVLGGDSEETLLPALTE

Comments.

1. The 215 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/7922 derived from phage 7922.
2. One region of 4 different amino acids (61-64) and 26 other amino acid differences were identified in antigenic polypeptide 28 as compared to an ORF encoded by bp 2940-3146 on ctg 167 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and to a related *M.tuberculosis* H37Rv ORF (the probable neuraminidase Rv3463; Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 29 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 29 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 29 comprises a DNA fragment of 206 bp derived from recombinant phage 2121. Phage 2121 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 29.

AATTATCGTCCTTCGGCTCGTCCTCGCAGTACGCCATTCCCGAACCAGTGCC
CGCATAGCGGGTTCCGTCGGCGTTGGTTCCCGGGGGCAGGGCATCGACGGT
CTGCTGGATGTAGTGGATCACTGTCTTCTGCGCTTCTCCTTGACTCGCAGGT
ATTTTCGGAGACGGGGCGGCTGGTGTCGATTCCATCGGACCTCCTGGAATT

Comments.

1. The 206 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/2121 derived from phage 2121.
2. The DNA sequence and the ORFS encoded by SEQ29 show no identity with the *M.bovis* AF 2122/97 genome and predicted ORF sequences (www.sanger.ac.uk/ Projects/M_bovis), nor with the genome and predicted ORF sequences of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 30 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 30 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 30 comprises a DNA fragment of 175 bp derived from recombinant phage 4925. Phage 4925 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 30.

AATTCCTCGGCGACGATGGCCACTTCGACACCCGAGGCGCCGTCGGAGCGC
AACGACCGCCAGCCGGACATCTCGATCTGCTTGTCCAGCCGGCTGATTCGG
CCCTGGTCGTCGAGATCCTGCACCGTTGCCGGGCCGAGGTCGTCGGCCAGC
TTGGCGGCGGCATCACGCAATT

Comments.

1. The 175 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/4925 derived from phage 4925.

2. The DNA sequence and the ORFS encoded by SEQ30 show no identity with the *M.bovis* AF 2122/97 genome and predicted ORF sequences (www.sanger.ac.uk/ Projects/M_bovis), nor with the genome and predicted ORF sequences of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 31 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 31 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ31 comprises a DNA fragment of 755 bp derived from recombinant phage 6924. Phage 6924 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 31.

AATTCGTGTTGGGTTGCCGCGCAGGCAATATGGACGTGCACCCCGACAGAT
GACCGGAGATCCGAGCGCATGAGCGAAGGCCAATACGGCACCTTCCACCTA
CCCCGGCTGGATTTTGCGAACGTTGCCGATGAGCGTCGATCGCGGTCTGGG
GTGGAAGACGTTGCGCGACGCCGGACCGGTGGTGTTCATGAACGGCCACTA
CTACCTCACCCGCCGGGAGGATGTGCTGGCGGCGCTGCGCAACCCGAAGGT
GTTCTCGTCGACGGTGCTGCAACCTCCCGGGCATCCGCTGCCGGTGCTGCC
GTTGGCATTTGACCCGCCGCAGCACACCCGCTATCGCAAAATCCTGCAGCCG
TATTTCAGCCCGCACGCGCTGGGCAAGTCCCGGCCGGTGCTGGAGCGCCAT
GCCGCAGAGATGATCGCCGCGTTGGCCGACCGTGGCGAGTGCGAAGTGATG
GCGGATTTCGCGCACCTGTATCCGTTCCAGGTGTTCATGGACCTCTACGGCC

TGCCGCTGCAGGATCGCGACCGCCTGCTCGACTGGAAAAACGCCGTCGTCG
GCGAGAAGCCGTTCGTCACCGAGTCCGACGTCGAGAAGTCCGAGCAACTGC
TGGCCTATCTCGCGGACGCGATCGCCCAGCGCCGGCAGCACCCCGGCACCG
ACATGCTGTCGCAGGTGATGACCGGCGAGGGCAACTTCACCGACATCGAAT
TGCTGGGAATGAGCCACCTGCTGATCCTGGCCGGGCTTG

Antigenic open reading frame encoded by SEQ31 – length 228 amino acids

MSEGQYGTFHLPRLDFATLPMSVDRGLGWKTLRDAGPVVFMNGHYYLTRRE
DVLAALRNPKVFSSTVLQPPGHPLPVLPLAFDPPQHTRYRKILQPYFSPHALG
KSRPVLERHAAEMIAALADRGECEVMADFAHLYPFQVFMDLYGLPLQDRDR
LLDWKNAVVGEKPFVTESDVEKSEQLLAYLADAIAQRRQHPXTDMLSXVMT
GEGNFTDIELLGMSHLLXLAGL

Comments.

1.The 755 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/6924 derived from phage 6924.

2.At least 4 regions of 4 or more different amino acids (164-167, 176-180, 186-189, and 209-213) and 57 other amino acid differences were identified in the antigenic polypeptide 31 as compared to an ORF encoded by bp 16029-16715 on ctg 249 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and to the related *M.tuberculosis* H37Rv cytochroom P450 (Rv1785c; Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 32 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 32 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 32 comprises a DNA fragment of 190 bp derived from recombinant phage 9121. Phage 9121 encodes *M. avium subsp. paratuberculosis* antigenic

polypeptide 32.

AATTGGCCTGGCCGGTCCCGCATTTCGCTGACCGTCTCGGCGATGCCGTGC
AGCGAGTAGTTGTTGCCCGGTCCGCCGAAGTACGGCAGGCCGCCGGTGAGC
GTCAGGCCGCGCGGATCGTCGGTGGCCAGGCCCGTGCCGTCGCAGAAGTTG
AACACCGGCACCGGGAAGCAGCTGTACAGATCGAACG

Antigenic open reading frame of SEQ 32 – length 62 amino acids

FDLYSCFPVPVFNFCDGTGLATDDPRGLTLTGGLPYFGGPGNNYSLHGIAETV
SEMRDRPGQ

Comments.

1. The 190 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/9121 derived from phage 9121.
2. Two regions of 4 or more different amino acids (12-18, and 51-59) and 17 other amino acid differences were identified in antigenic polypeptide32 as compared to an ORF encoded by bp 27852-28037 on ctg 247 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and to the related *M.tuberculosis* H37Rv possible actetyl CoA synthase (Rv1867; Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 33 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 33 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ33 comprises a DNA fragment of 744 bp derived from recombinant phage 7921. Phage 7921 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 33.

AATTCGTCTGGGGCCGCCAGTACGGGGAGAACACGATCAACCCATGAGAAT

CACCGGTACCGCAGTCAAACTCGTCGTCTTCTGGTCCGTGCTGGCGATGTTC
ACAGTGATGATCATCGTCGTGTTCGGCCAGGTCCGATTCGATCGGACCACCG
GCTACTCCGCGGTGTTCACCGACGCCGGCGGGTTACGGGCCGGGCAGTTCG
TGCGCGCCTCCGGGGTGGAGGTGGGCAAGGTCGCCGCCGTGACGCTTTCCG
ACAAGGACAGCCGGGTGTTGGTGGAGTTCAACGTGGATCGCTCACTGGCAC
TGGACCAGGGCACCACCGCGTCGATCCGCTACCTCAACCTGATCGGCGACC
GCTATCTGGAACTCAAGCGCGGCACCAGCGGCCGCCGGCTGCCCCCGGGTG
GCCGCATCCCGGTCGAGCACACTCAGCCGGCGTTGGATCTCGACGCGCTGA
TCGGCGGATTCCGGCCGCTGTTCCAGGCTTTGGACCCGAACAAGGTCAACA
GCATCGCCCAGTCCATCATCACCGTGTTCCAGGGACAGGGCGCCACCATCAC
CGACATCCTCGACCAGACCGCGGCGCTGACCGCCGCGCTGGCCGACCGCGA
CAAGGCGATCGGCGAGGTGATCAACAACCTGAACACCGTGCTGGCCACCAC
CGTCAAGCACGAGAANGAGTTCGACCGAACGGTCGACAAGTTGGAACTGCT
GATCACCGGATTGAAGAACCCGGGCCG

Antigenic open reading frame encoded by SEQ 33 – length 247 amino acids

IRLGPPVRGEHDQPMRITGTAVKLVVFWSVLAMFTVMIIVVFGQVRFDRTTGY
SAVFTDAGGLRAGQFVRASGVEVGKVAAVTLSDKDSRVLVEFNVDRSLALDQ
GTTASIRYLNLIGDRYLELKRGTSGRRLPPGGRIPVEHTQPALDLDALIGGFRP
LFQALDPNKVNSIAQSIITVFQGQGATITDILDQTAALTAALADRDKAIGEVIN
NLNTVLATTVKHEXEFDRTVDKLELLITGLKNPG

Comments.

1. The 744 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/7921 derived from phage 7921.

2. Fourty six amino acid differences were identified in the antigenic polypeptide33 as compared to an ORF encoded by bp 23706-24440 on ctg253 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and a related *M.tuberculosis* H37Rv hypothetical protein (Rv0590; Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 34 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 34 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ34 comprises a DNA fragment of 590 bp derived from recombinant phage 6222. Phage 6222 encodes *M. avium subsp. paratuberculosis* antigenic polypeptide 34.

AATTCGGCTTCGTAGACGGCGTCGGGAATCTTCACCGCGGCCCGGGCCGAT
TTCTTCTTGCCGGCCTTGAGGGGAACGCCGTCATTTTTCGCGCTGCTCACAG
CCACGAATGATAGCCCCACGCAGGGATTTTCAGCGCTGTCTAGCGCTGCCTA
AGACCGGGCGGCCTCCACCAGTTCCTCGAGCGCCTTGGTTCAGCAATGCGG
GCAGCAGGTCGACGTCGCTCATCGCTTCGCGGTCGGCGNTGATGCCGTAGT
AGAGCATGCCGNTGTAGGACGTCACGCNGATGGCCAGCGCCTGGTTGTGCA
GCAGCGGCGGCACCGAGTAGGTCTCCAGCAGCTTGGTGCCGGCGACGTACA
TCTGCGACTGCGCCCCGGGCGCGTTGGTGATCAACAGGTTGAAGGTGCGCT
TGGAGAAGCTCGGGAAGCTGGTCGCGACCCGGATGCCCATGGCGTGCAGGG
TGGGCGGGGCGAAACCCGACAGCGTGACGATGGTCCCGGCGTCCACCAGAT
GCGCGGCCGCCGGGCTGGATTCGGCGGCGTGCGCGATCTGGGAGAGCCGC
ACCACCGCGTTCGGCTCCCCCACCGGCC

Antigenic open reading frame by SEQ 34 – length 35 amino acids

VAVSSAKNDGVPLKAGKKKSARAAVKIPDAVYEAE

Comments.

1. The 590 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/6222 derived from phage 6222.
2. The DNA sequence and antigenic polypeptide 34 show no identity with the *M.bovis* AF 2122/97 genome and predicted ORF sequences (www.sanger.ac.uk/

Projects/M_bovis), nor with the genome and predicted ORF sequences of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 35 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 35 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ 35 comprises a DNA fragment of 625 bp derived from recombinant phages 4112111. Phage 4112111 (and 11 other phages) encode *M. avium subsp. paratuberculosis* antigenic polypeptide 35.

AATTCGCGCATACCCGTCACTGGTCACAACGCCACATGCTGGTAGGCTGT
GGAATCGAGGGTCAATCCGGATCGGACCCCAACGTCGACTTGTGGGCGCC
AATTCGCGGGTTTTCGCCCAGCAAGTCGACGTTCGGCGCGAATCGGTGAG
GTGGGCACAGGTGAATGACGAAGAGGACATGCTGGTCGCCACGGTGCGG
GCGTTCATCGACCGCGAGGTCAAACCGACCGTGCGCGAGGTGGAGCACGC
CGATGCCTATCCCGAGGCGTGGATCGAGCAGATGAAGCGGATCGGGATCT
ACGGGCTGGCGGTGCCCGAGGAATACGGTGGTTCGCCGGTGTCCATGCCG
TGCTACGTGCGGGTCACCGAGCAGCTGGCGCGCGGCTGGATGAGCCTGGC
CGGGGCGATGGGCGGGCACACCGTGGTGGCCAAGCTGCTAACGCTGTTCG
GCACCGAGGACCASAAGCGGGCCTACCTGCCGCGGATGGCCAGCGGCGA
AATCCGGGCCACCATGGCGTTGACCGAGCCCSGCGGCGGCTCGGACCTGC
AGAACATGTCGACCACCGCGCTGCCCGACCCCGACTCCGACGGNCTGGTG
GTCAACGGGGCCAAGACCTGNATCAAC

Antigenic open reading frame by SEQ 35 – length 117 amino acids

MLVATVRAFIDREVKPTVREVEHADAYPEAWIEQMKRIGIYGLAVPEEYGGS
PVSMPCYVRVTEQLARGWMSLAGAMGGHTVVAKLLTLFGTEDXKRAYLPR
MASGEIRATMALTEP

Comments.

1. The 625 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/4112111 derived from phage 4112111.
2. Fifteen amino acid differences were identified in the antigenic polypeptide 35 as compared to an ORF encoded by bp 18838-19188 on ctg 213 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and to the related *M.tuberculosis* H37Rv FadE3 protein (Rv0251c; Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 36 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 36 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ36 comprises a DNA fragment of 241 bp derived from recombinant phages 4422111 and 4422112. Phage 4422111 (and 4422112) encode *M. avium subsp. paratuberculosis* antigenic polypeptide 36.

GTGGGGGCAAGCCAATTACGTTCGCATCGACCCGGCACAGGCGGTCGCTC
ACGTCATCAACATGCCGCTCATCCCCGATGAGGCTCGAATGACCTTGCTAC
GCAGGCGCTGAACGCACGACGAAACGGACCGGAGGTGAAAGGGACATGA
GCCACGCCGATCAACTCGCTCGGACGCACCTGGCGCCCGATCCTGCGGAC
CTGTCGCGCCTGGTCGCCGGCACCCACCACGACCCGCACGG

Antigenic open reading frame by SEQ 36 – length 31 amino acids

MSHADQLARTHLAPDPADLSRLVAGTHHDPH

Comments.

1. The 241 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/4422111(and pTriplEX4422112) derived from phage 4422111 and 4422112.

2.At least 1 region of 4 or more different amino acids (3-7) and 8 other amino acid differences were identified in the antigenic polypeptide 36 as compared to a ORF encoded by bp 2559-2651 on ctg 276 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and to the related *M.tuberculosis* H37Rv glgb protein (Rv1326c; Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 37 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 37 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ37 comprises a DNA fragment of 236 bp derived from recombinant phages 4722141 and 4722142. Phages 4722141 and 4722142 encode *M. avium subsp. paratuberculosis* antigenic polypeptide 37.

GGACACCAACGTGACCGGGGTGTTTCTCACCGCCCAGGCGGCGGCCCGGG
CGATGATGCGGCAGGGCCGCGGCGGCGCCATCATCACCACCGCCTCGATG
TCCGGGCACATCATCAACGTCCCGCAGCAGGTCGGCCACTACTGCGCCAG
CAAGGCGGCCGTGATCCAGCTGACCAAGGCCATGGCCGTCGAATTCTGCA
GGATCCGTCGACTCTAGACTCGAGCAAGCTTATGCA

Antigenic open reading frame by SEQ 37 – length 70 amino acids

NVTGVFLTAQAAARAMMRQGRGGAIITTASMSGHIINVPQQVGHYCASKAA
VIQLTKAMAVEFCRIRRLX

Comments.

1. The 236 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/4722141 (and pTriplEx/4722142) derived from phage 4722141 and 4722142.

2. Eight other amino acid differences were identified in the antigenic polypeptide 37 as compared to an ORF encoded by bp 1360-1545 on ctg 265 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and to *M. tuberculosis* H37Rv ORF (Rv1928c; Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 38 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 38 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ38 encodes a DNA fragment of 419 bp derived from recombinant phages 4222121 and 4222122. Phages 4222121 and 4222122 encode *M. avium subsp. paratuberculosis* antigenic polypeptide 38.

CGGCCACCGCACCCAGGGGAGGCCATGACTCACACCAAGGCCGGTCGTGC
CGCGTGGCCGGCCGCCTGCGCGGTCGTCCTGTCCGCCGCCGCGCTGTTGTG
CGCGGCAGCGGCCGCCGCGGACGAAGCCGATGACGCGTTCCTCGCCGGCC
TGGCCAAGGGCGGGATCACCATGTTCGACGACGACGACGCGATCGCCATG
GGCCACAGCGTGTGCTCGAGCATCGACGCCAACCCCAACGTGTCGATGCT
GGCGCTGCGGCTGACCAAGCAAACCCCGTTGACGCCGAAGCAATCCGGCT
ACTTCATCGGTCTTTCGGTCGCCAGCTACNTGCCCGCAGTACAAGGACGA
CGTCGACCCCTCGCTGGGCTGGCTGATCCCGCCGCCGCTGATGTGANGTTG
CCGGCCGGCATCGGCGT

Antigenic open reading frame by SEQ 38 – length 125 amino acids

MTHTKAGRAAWPAACAVVLSAAALLCAAAAAADEADDAFLAGLAKGGITM
FDDDDAIAMGHSVCSSIDANPNVSMLALRLTKQTPLTPKQSGYFIGLSVASYX
PAVQGRRRPLAGLADPAAADVX

Comments.

1.The 419 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/4222121 (and pTriplEx/4222122) derived from phage 4222121 and 4222122
2. The DNA sequence and antigenic polypeptide 38 show no identity with the *M.bovis* AF 2122/97 genome and predicted ORF sequences (www.sanger.ac.uk/Projects/M_bovis), nor with the genome and predicted ORF sequences of *M.tuberculosis* H37Rv (Cole et al. 1998. Nature 393:537-544).

The invention provides an isolated and/or recombinant nucleic acid comprising SEQ 39 or functional fragments thereof, a host cell comprising said nucleic acid, and an antigenic (poly)peptide and a fragment encoded by SEQ 39 or fragments thereof as described hereunder and an isolated or recombinant nucleic acid essentially encoding such an antigenic (poly)peptide or fragment thereof.

SEQ39 comprises a DNA fragment of 392 bp derived from recombinant phages 4622122 and 4622121. Phages 4622122 and 4622121 encode *M. avium subsp. paratuberculosis* antigenic polypeptide 39.

CGGCGTAGCATCGTCAAGTCGTTGCCCGCGCTGATGCCGGAGCGGCAGTA
AGGAGTTCGGCTGGTGCAAAAACGCTTGCCCACAGTCGTTTTGGTGCTGA
CGGCCGTTGTCGCCGGTATCGCCGGGTGCAGCGCGGCGCAGACCGTGCCG
CGCAAGGCCGCCCGGCTGACCATCGACGGTGCCACCCACACGACCCGCCC
GCCGTCCTGCCGGCAGGACCAGATGTATCGGACCATCAACATCCCCGACC
ACGACGGTGGAGTCGAAGCGGTGGTGCTGCTCAGCGGTTACCGGGTGATG
CCGCAGTGGGTGAAGATCCGGAACGTCGACGGCTTCACCGGCAGTCTACT
GGCCASGGCGGAGTGGGCGACGCGCACGTCGATCTCACMAAT

Antigenic open reading frame by SEQ 39 – length 101 amino acids

GVRLVQKRLPTVVLVLTAVVAGIAGCSAAQTVPRKAARLTIDGATHTTRPPSC
RQDQMYRTINIPDHDGGVEAVVLLSGYRVMPQWVKIRNVDGFTGSLLA

Comments.

1.The 392 bp DNA sequence was obtained by sequence analysis of plasmid pTriplEx/4622122 (and pTriplEx/4622121) derived from phage 4622122 and 4622121.

2. 29 amino acid differences were identified in the antigenic polypeptide 39 as compared to an ORF encoded by bp 51316-51600 on ctg 265 of the *M.bovis* AF 2122/97 genome sequence (www.sanger.ac.uk/Projects/M_bovis), and to the related *M.tuberculosis* H37Rv cytochroom lppE protein (Rv1881c; Cole et al. 1998. Nature 393:537-544).

Examples of use.

The currently available validated serodiagnostic and cell-mediated immune have a relative good specificity in the detection of a specific immune response to *M avium subsp. paratuberculosis* but clearly are not sensitive enough to be useful in detection and control of paratuberculosis. A problem that is solved here is therefore the development of immunodiagnostic tests for paratuberculosis with an improved sensitivity, particularly useful for early diagnosis of paratuberculosis, by detecting a differentiating immune response against one of above identified specific antigenic peptide fragments, comprising or consisting of a peptide fragment essentially derived from *M. avium subsp. paratuberculosis* bearing essentially a functional, or at least an antigenic, difference to a *M. bovis* and/or *M. tuberculosis* antigen. Of course, nucleic acid based differential diagnostic techniques are now also provided for differentiating diagnostic use.

The currently approved vaccines are all based on whole bacteria and display fair protection to clinical paratuberculosis, but do however interfere with the immunodiagnosis of bovine tuberculosis, still an important veterinary problem in many countries. We therefore provided here improved (subunit)vaccines that do not or only neglibly induce cross-immunity to bovine tuberculosis. In particular the genes encoding the antigenic polypeptides 1, 2, 7, 18, and fragments thereof are useful. To establish the usefulness of these antigens for inclusion in an immunodiagnostic assay and for preparation of a subunit vaccine for inducing protective efficacy, these antigenic polypeptide 1, 2, 7, 18, were over-expressed and purified as recombinant proteins. Hereto, we expressed the antigenic polypeptides as poly-histidine tagged fusion proteins in *E.coli* XL1Blue by cloning DNA fragments, obtained by PCR amplification using specific primers and genomic DNA from *M. avium paratuberculosis* strain B854, into the vectors pQE80, pQE81 and pQE82 (obtained from QIAGEN) by employing standard molecular biological procedures known to individuals skilled in the art. Briefly, the amplified DNA fragments encoding antigenic polypeptide 1, 2, 7, 18, 19 and 20 were cloned into the vector PCR4-TOPO (Invitrogen) according to the manufacturers instruction manual; The inserted DNA fragments encoding antigenic polypeptide 1, 2, 7, 18 were subsequently confirmed by DNA

sequencing, and subcloned into the expression vectors PQE80-82 series. Expression of the now his-tagged antigenic polypeptides 2, 7, 18 was visualised by Coomassie Brilliant Blue staining of SDS-PAGE, and by western blotting with serum 3869 (antigenic polypeptide 1, 2 and 7) or with the respective monoclonal antibodies reactive with antigenic polypeptide 18. The recombinant antigenic polypeptides 1, 2, 7, 18 were subsequently purified to a purity of > 95 % (as detected by Coommassie Brilliant Blue stained SDS-PAGE) by immobilised metal-chelate affinity chromatography according to the QIAGEN manual for purification ("The QIAexpressionist"; fourth edition-january 2000). Contaminating *E. coli* lipopolysaccharide was removed to a level of < 50 EU/mg protein by affinity chromatography using Detoxi-Gel Endotoxin Removing Gel (Pierce) according to the protocol provided by the manufacturer. The thus antigenic polypeptides retained their recognition by serum 3869 (antigenic polypeptide 1, 2 and 7) or by the respective monoclonal antibodies reactive with antigenic polypeptides 18 both as tested by immuno-blotting and by dot-blotting.

The serodiagnostic relevance of the purified antigenic polypeptides 1, 2, 7, 18, is tested by evaluating particularly their sensitivity (but also their specificity) both in a direct ELISA format and in an ELISA inhibition format using specific monoclonal antibodies. Hitherto we use panels of serum samples (but also tissue and body fluid samples such as for example whole blood and milk) obtained from ruminants, more specifically cows, sheep and goats, that are naturally or experimentally infected with *M. avium subsp. paratuberculosis*, and in various, particularly early, stages of infection. These antigenic polypeptides prove in principle particularly sensitive (and specific) as compared to currently used and validated absorbed ELISA test but may be further investigated for the purpose of improving and validating the particular serodiagnostic format, with appropriate methods known in the art, but also by evaluating these antigenic polypeptides in a variety of other serodiagnostic formats including single step lateral flow "dipstick" formats. If indicated, and to improve sensitivity and specificity also specific subfragments or deleted forms of antigenic polypeptides 1, 2, 7, 18, be produced by cloning and expression of truncated forms of the genes encoding antigenic polypeptides 1, 2, 7, 18, 19, and 20, or by introducing specific deletions into the genes encoding antigenic polypeptides 1, 2, 7, 18 by the procedures described above. Again, (a combination of) these products are evaluated for

sensitivity (and specificity) in the above indicated serodiagnostic formats and compared with the validated absorbed ELISA assay.

The diagnostic relevance of the purified antigenic polypeptides 1, 2, 7, 18 in cell-mediated assays will be further tested by evaluating particularly their sensitivity (but also their specificity) initially in a whole blood format where secretion of interferon-gamma is detected by comparing with the validated BOVIGAM assay. We will use whole blood samples obtained from ruminants, more specifically cows, sheep and goats, naturally or experimentally infected with *M. avium subsp. paratuberculosis*, in various, particularly early, stages of infection. (A combination of) those antigenic polypeptides proving particularly sensitive (and specific) as compared to currently used and validated BOVIGAM test will be further investigated by improving and validating the particular format, but also by evaluating other *in vitro* cell culture formats including for example by use of cell proliferation as a read out. If indicated, and to improve sensitivity and specificity also specific subfragments or deleted forms of antigenic polypeptides 1, 2, 7, 18 can be produced by cloning and expression of truncated forms of the genes encoding antigenic polypeptides 1, 2, 7, 18 or by introducing specific deletions into the genes encoding antigenic polypeptides 1, 2, 7, 18 by the procedures described above. Again, these products are evaluated for sensitivity (and specificity) in the above indicated cell-mediated assay formats and compared with the validated BOVIGAM assay.

To evaluate the usefulness of the purified polypeptides 1, 2, 7, 18 for developing improved vaccines that do not interfere with immunodiagnosis for bovine tuberculosis, subunit vaccines are prepared from the host cells provided herein based on the purified antigenic polypeptides mixed with strong adjuvants (an oil-based emulsion is particularly useful), immunise goats and evaluate the induction of skin-test reactivity with bovine and paratuberculosis derived PPDs. (A mixture of) those subunit vaccins positive with paratuberculosis PPD, but negative with bovine PPD are evaluated for protective efficacy by comparing the protective efficacy with currently available whole cell based vaccines using the goat model. In addition, (a mixture of) of (sub)vaccines based on other adjuvants

and based on DNA vaccines carrying the genes encoding the antigenic polypeptides 1, 2, 7, 18, are tested. The protective efficacy resembles or exceeds the efficacy that is achieved by a currently available whole cell vaccine but a vaccine according to the invention has the added advantage that it does not interfere with immunodiagnoses of (bovine) tuberculosis.

Claims

1. A method for obtaining a recombinant host cell comprising an antigenic polypeptide fragment of *M. avium subsp. paratuberculosis* and its encoding nucleic acid comprising providing an recombinant expression library of host-cells expressing *M. avium subsp. paratuberculosis* nucleic acid and immunoscreening said library with a serum obtained from a ruminant infected with *M. avium subsp. paratuberculosis* but not with *M. bovis*, said serum essentially obtained from a late stage of infection of said ruminant with said Mycobacterium, the method further comprising selecting a host cell that expresses an antigenic peptide fragment that is immunoreactive with said serum.

2. A method according to claim 1 wherein said ruminant is a cow.

3. A host cell obtainable by a method according to anyone of claims 1 to 2.

4. An antigenic polypeptide comprising a peptide fragment essentially derived from *M. avium subsp. paratuberculosis* bearing essentially no functional homology to *M. bovis* and/or *M. tuberculosis.*

5. A peptide according to claim 4 obtainable from a host cell according to claim 3.

6. A nucleic acid encoding a peptide according to claim 4 or 5

7. An antibody directed against a peptide according to claim 4 or 5.

8. Use of a peptide according to claim 4 or 5 and/or a nucleic acid according to claim 6 and/or an antibody according to claim 7 in a method for testing samples for detecting *M. avium subsp. paratuberculosis* infections.

9. A diagnostic test kit for the detection of *M. avium subsp. paratuberculosis* infections comprising a peptide according to claim 4 or 5 and/or a nucleic acid according to claim 6 and/or an antibody according to claim 7.

10. Use of a host cell according to claim 3 and /or a fragment according to claim 4 or 5 and/or a nucleic acid according to claim 6 for the preparation of a vaccine for the treatment or prevention of *M. avium subsp. paratuberculosis* infections.

11. A vaccine for the treatment or prevention of *M. avium subsp. paratuberculosis* infections comprising a host cell according to claim 4 and /or a fragment according to claim 4 or 5 and/or a nucleic acid according to claim 6.

Abstract

The invention relates to the field of diagnosis, treatment and prevention of Johne's disease. The invention provides a method for obtaining a recombinant host cell comprising an antigenic polypeptide fragment of *M. avium subsp. paratuberculosis* and its encoding nucleic acid comprising providing an recombinant expression library of host-cells expressing *M. avium subsp. paratuberculosis* nucleic acid and immunoscreening said library with a serum obtained from a ruminant infected with *M. avium subsp. paratuberculosis* but not with *M. bovis*, said serum essentially obtained from a late stage of infection of said ruminant with said Mycobacterium, the method further comprising selecting a host cell that expresses a fragment that is immunoreactive with said serum.

FIGURE 1